# PAEDIATRIC DYSLIPIDEMIA AS A PREDICTOR OF DYSLIPIDEMIA AND CAROTID ARTERY THICKENING IN YOUNG ADULTS

## Findings from the Childhood Determinants of Adult Health (CDAH) Study, the Cardiovascular Risk in Young Finns Study, and the Bogalusa Heart Study


by

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*To Jayden, Brocklan, Noah, and Jaymee*

*To Graham, Yvonne, Kym, and Kerri*

*To Charlotte*

*To the one who never was and never will be*
*Your words inspired me*
*Your enthusiasm made me feel alive*
*You opened my eyes to a different world*
*You made me want to be... a better man*

# DECLARATION OF ORIGINALITY

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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# CONTRIBUTION OF THE AUTHOR TO THE CDAH FOLLOW-UP SURVEY

A team of academic, administrative, and fieldwork staff contributed to the development and implementation of the Australian Childhood Determinants of Adult Health (CDAH) follow-up survey. As part of this team, the author (CM) provided several contributions that included:

*Study Development*

- Development of: fitness protocols (cardiorespiratory, muscular strength, and muscular power); fitness equipment maintenance and quality control protocols; and fitness screening questionnaire.
- Primary person responsible for development, recruitment, collection of data, and data analysis for study to assess the validity of vascular measures derived from a portable ultrasound machine earmarked for use in the CDAH study.
- Contributions to vascular ultrasound protocol development and reviewing possible measures for inclusion in the study.
- Trained to perform ultrasound examinations and act as reserve sonographer if required. Ultrasound training included: time spent with local technicians, time spent with technician on secondment from the Cardiovascular Risk in Young Finns Study; one week with clinicians at the Royal Prince Alfred Hospital Sydney; and over 50 practice studies performed.
- Contributions to successful grant application (Tasmanian Community Fund, D0013808) that secured funding for ultrasound equipment, C-reactive protein and fibrinogen assaying, and angiotensin-converting enzyme genotyping.
- Contribution to CDAH pilot study and the pilot review process.

*Data Collection*

- Training of field staff to perform fitness measures.

- Being a member of the data collection team in Tasmania and Western Australia. Tasks included: cardiorespiratory fitness exam, muscular strength and power assessment, lung function tests, and pedometer issue.
- Primary person responsible for vascular measurements from ultrasound images.

*Data Entry, Management, and Analysis*

- Ongoing contributions to data entry, data analysis and data management decisions involving key measures including: cardiovascular risk factors; ultrasound measures; metabolic syndrome; and fitness.

# CONTRIBUTION OF THE AUTHOR TO THE THREE STUDY COLLABORATION

This thesis makes use of data from three prospective cohort studies that commenced in childhood and adolescence: the Childhood Determinants of Adult Health (CDAH) study, the Cardiovascular Risk in Young Finns Study, and the Bogalusa Heart Study. While support for research collaboration was initiated at a meeting between the chief investigators of the CDAH and Young Finns studies and by correspondence from the chief investigator of the Bogalusa Heart study in 2001, the author conducted all negotiations to collaborate on this specific project with the Young Finns and Bogalusa research groups who then provided data for this thesis; the first of the data pooling exercises between the three cohorts. CM obtained the data, prepared them for analysis, developed the analysis plan, and conducted the analysis. The thoughts and ideas for the direction of this project were identified by CM as being of likely clinical and scientific importance.

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This dissertation was carried out at the Menzies Research Institute, University of Tasmania, Australia from 2003 to 2009 using data from the Childhood Determinants of Adult Health (CDAH) Study, the Cardiovascular Risk in Young Finns Study, and the Bogalusa Heart Study. I am grateful to the former Director **Professor Terry Dwyer** and the current Director **Professor Simon Foote** of the Menzies Research Institute for allowing me the support of the institute's facilities and faculty over the tenure of my candidature. During my time at Menzies, several people at the institute have helped me to learn the intricacies of modern epidemiology and biostatistics, supported my ambitions, and have pushed me to reach greater research and personal heights. I express my sincerest gratitude to CDAH project manager **Ms Marita Dalton**, CDAH staff and volunteers (past and present), **Ms Jane Fryer** for statistical advice, **Professor Emeritus David W. Hosmer** and wife **Trina Hosmer**, **Associate Professor C. Leigh Blizzard**, **Dr Velandai Srikanth** – always a source of motivation, **Dr Robert Granger** – for your appreciated support and ability to make things happen, **Dr Michael D. Schmidt** – always a sounding board for my ideas and thoughts, and **Dr Verity Cleland** – the trailblazer.

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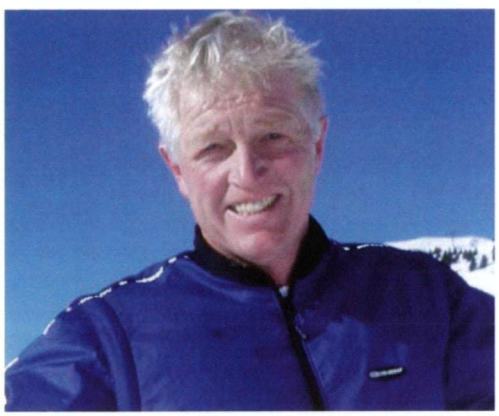
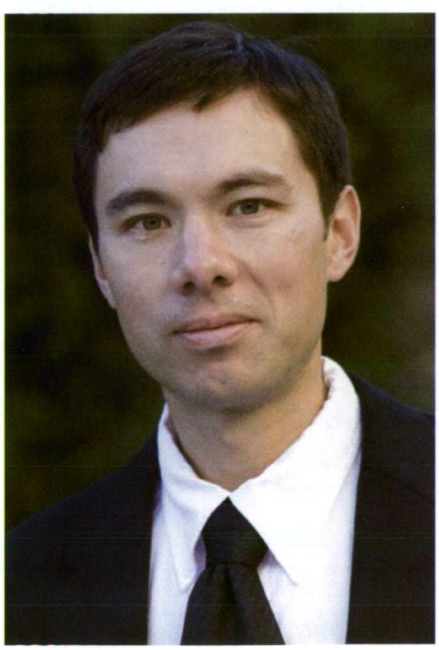
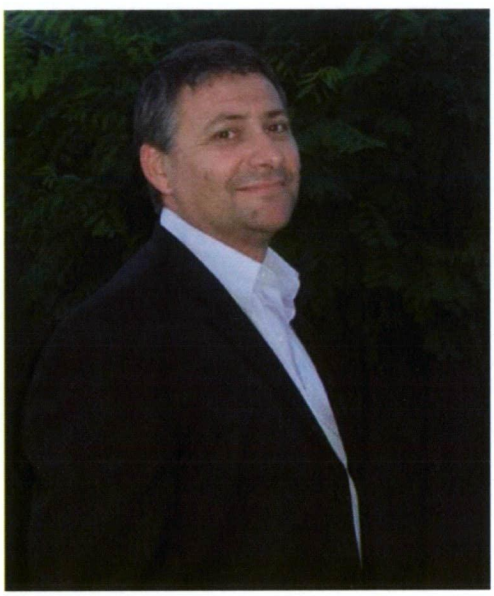


**Peter Rehor, PhD -** Associate Professor Peter Rehor initiated my interest in research with his absorbing and passionate lectures on an arguably uninteresting subject (research methods) in the second semester of the first year of my undergraduate studies. For the first time, a personally viable career choice that had the potential to be both dynamic and rewarding appeared to me. The stark contrast in grades between my first and subsequent years of my undergraduate studies could be loosely attributed to the influence your lectures had on me, and the emerging desire to pursue a research career. If it were not for your passion in research and your ability to transfer this passion to your students, I may not find myself in this position.



**Trevor Innes, PhD** – Dr Trevor Innes (the wood doctor) became somewhat of an idol to me. We met through a shared interest in cycling but at a time when my curiosity in research had begun to develop. I watched as you balanced your professional life with your passion for cycling. You had the expertise and knowledge that could afford you to pursue a research career part-time while racing at the premier

grade on the state and national cycling scene. This lifestyle was attractive to me. As an observer, this gave me continued motivation to pursue a career in research. However, more than simply illustrating to me some of the potential opportunities of a research career, I appreciated and continue to value the friendship we developed during this period – mostly built-up over endless training hours in the saddle. I look forward to catching up with you in the near future and I thank you for being instrumental in facilitating my research career.



**P. Dean Cooley, PhD** – As a lecturer, Dr Dean Cooley had the reputation of being an excessively hard assignment marker. As an honours supervisor, he was known to have the highest ratio of first-class honours graduates of any staff member in the Centre for Human Movement (nine in the previous 10 years). It was for these reasons that I approached him to be my honours supervisor in 2002. Although having Dean as my supervisor necessitated I change my project field from exercise physiology to sport psychology, this decision proved to be the correct one. Dean was instrumental in developing my research knowledge particularly with regard to statistics, scientific rigor, and writing. Through Dean's guidance I was able to achieve first class honours, which would prove important in securing a PhD position and scholarship. One of the most important attributes Dean instilled in me was the ability, through self-motivation, to seek unknown knowledge and continue the learning process past the formal training years. While Dean remains a mentor and sounding board for my research ideas, I consider him a dear friend – a person I always look forward to catching up with. I wish you and your family all the best for the future and look forward to many more bicycle rides where we vent shared frustrations with the world, and kicking back with a beer at the Launceston criterium at Christmas.

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**Alison Venn, PhD** – My earliest recollection of Associate Professor Alison Venn was at the interview for my PhD scholarship. I remember sitting rather perplexed as Alison conversed about the CDAH (pronouncing as *cedar*) study, and me, none the wiser, trying to work out exactly what cedar meant while remaining on top of the questions being asked of me. Confusion however, was not to set the scene accurately for the time to follow. Through my earliest days in my PhD, I struggled to understand just why Alison was so enthused with epidemiology. Despite my sometimes wavering motivation and my innate ability to change focus often, Alison was always supportive, something I would liken to a parent giving room to allow a child to discover the world for oneself, but providing guidance when asked upon (or when otherwise needed). While my peers have often commented on my determination, this is a product of two necessary ingredients. The first is that I am motivated by something intrinsically meaningful, which I need to discover for myself; the second is the need to have people who are supportive behind me. Alison perceived this in me and supplied me an environment that included these two components. While I am appreciative of the space and guidance you provided me, it has been my more recent work with new research students at the Institute that has made me aware of the impact of your teachings. The knowledge and guidance you once shared with me has now become the knowledge and guidance I offer to these new students. And now, when I get enthused about the potential of a study or research question in epidemiology, these students look at me with the same non-impressed gaze that I once gave to you. It would seem then, that my perspective has changed in the time of my candidature. I wish to express my deepest gratitude and appreciation for your mentoring over these past years. I can now honestly say that I share your enthusiasm for epidemiology, and I look forward to working with you on papers and initiatives in the future.

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*And then the quiet, unassuming one seised the knife and carved his initials on the paper-bark tree. Troubled by his actions he dropped the knife at his feet and looked to the sky. There were no stars tonight, or so it seemed. The others stood silently, paralysed with fear. Then, the ground began to shake and a crack appeared in the surface. It spread quickly, forming a crevice that divided him and the paper-bark tree from the others. Much time would pass before he would realise the entire complexity of his actions that night.*

*...My friend with angels and demons*
*Trust in your past*
*Because from it*
*A great man has grown*

Costan G. Magnussen
Hobart, 2009

# ABSTRACT

**Background:** There is growing evidence that atherosclerosis begins at an early age and progresses silently throughout the life span before clinical complications such as myocardial infarction and stroke present. Adverse blood lipid levels (dyslipidemia) are an important contributor to the disease and have been the target of consensus statements for paediatric screening and treatment issued by the American Academy of Pediatrics and the US National Cholesterol Education Program since 1992; the most recent update being July 2008. The basis of these documents is that treatment of lipid disorders beginning in childhood or adolescence may reduce the lifetime risk of atherosclerotic cardiovascular disease.

**Aims:** This study examined: tracking of blood lipid levels from childhood to adulthood; the utility of two paediatric dyslipidemia classifications that define normal-, borderline-, and high-risk lipid blood levels to predict dyslipidemia and a measure of preclinical atherosclerosis (arterial wall thickness) in adulthood; factors that both reduce and improve prediction of those with dyslipidemia or arterial wall thickening in adulthood; whether changes in blood lipid levels between adolescence and adulthood had an effect on the level of arterial thickening measured in adulthood.

**Methods:** This study utilised data from three population-based prospective cohort studies from Australia (Childhood Determinants of Adult Health Study), Finland (Cardiovascular Risk in Young Finns Study), and the United States (Bogalusa Heart Study). Baseline data were collected on children and adolescents aged 9-18 years in 1980-1986. Between 2001 and 2006 participants from the original cohorts, now young adults aged 28-39 years, were re-examined (follow-up). Participants from each cohort had risk factor data (including fasting blood lipids) measured at both time-points. At follow-up, each cohort used carotid artery ultrasound to determine arterial wall thickness.

**Results:** The findings showed that paediatric dyslipidemia classifications predicts dyslipidemia and arterial thickening in young adulthood; that the classifications examined differ marginally in how strongly they predict adult dyslipidemia but perform with equal success in the prediction of arterial thickening in adulthood; that universal (population-wide)

or selective screening approaches that use paediatric dyslipidemia classifications would be limited by either high rates of false positives or high rates of false negatives; that adolescent lipid levels are more strongly associated with arterial thickening in adulthood than change in lipid levels; that dyslipidemia in the presence of overweight or obesity places affected adolescents at substantially higher risk of increased arterial thickening as adults; and that participants identified as high-risk in childhood but who adopted positive changes in lifestyle habits in the intervening years were less likely to have dyslipidemia as adults.

**Conclusions:** These findings suggest that paediatric dyslipidemia classifications are useful in predicting adolescents who are at increased risk of having dyslipidemia or preclinical atherosclerosis in young adulthood. While these findings suggest there would be limitations in screening the general paediatric population for dyslipidemia, they underscore the importance of both population-wide and individualised prevention programs to reduce the early development of atherosclerosis associated with paediatric dyslipidemia.

# CONTENTS

# LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications. Some communications provide additional information/analyses that are not central to the aims of this thesis and have not been included in the associated chapters. In addition, un-published data central to the thesis' aims are presented herein where appropriate. The following original contributions are ordered as they are cited in the thesis and are provided at the end of this document.

I **Magnussen CG**. Omissions from new lipid screening guidelines. Comment on Daniels, Greer, and the Committee on Nutrition. Lipid Screening and Cardiovascular Health in Childhood. Pediatrics 2008; 122: 198-208. Published online at *Pediatrics* on December 24 2008.

II **Magnussen CG**, Fryer J, Venn A, Laakkonen M, Raitakari OT. Evaluating the use of a portable ultrasound machine to quantify intima-media thickness and flow-mediated dilatation: Agreement between measurements from two ultrasound machines. *Ultrasound in Medicine and Biology.* 2006; 32(9): 1323-1329.

III **Magnussen CG**, Thomson R, Cleland VJ, Dwyer T, Venn A. Factors affecting the stability of blood lipid and lipoprotein levels from childhood to adulthood: Evidence from the Childhood Determinants of Adult Health (CDAH) study. Submitted to Pediatrics.

IV **Magnussen CG**, Raitakari OT, Thomson R, Juonala M, Patel DA, Viikari JSA, Marniemi J, Srinivasan SR, Berenson GS, Dwyer T, Venn A. Utility of currently recommended pediatric dyslipidemia classifications in predicting dyslipidemia in adulthood: Evidence from the Childhood Determinants of Adult Health (CDAH) Study, Cardiovascular Risk in Young Finns Study, and Bogalusa Heart Study. *Circulation.* 2008; 117(1): 32-42.

V **Magnussen CG**, Venn A, Juonala M, Thomson R, Viikari JSA, Srinivasan SR, Berenson GS, Dwyer T, Raitakari OT. The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood: Evidence from the Cardiovascular Risk in Young

Finns Study, the Bogalusa Heart Study, and the CDAH (Childhood Determinants of Adult Health) Study. *J. Am. Coll. Cardiol.* 2009; 53(10): 860-869.

VI **Magnussen CG**, Thomson R, Juonala M, Viikari JSA, Dwyer T, Raitakari OT, Venn A. Use of B-mode ultrasound to examine preclinical markers of atherosclerosis: Does image quality bias associations between cardiovascular risk factors and measures of vascular structure and function? To be submitted to Atherosclerosis.

# ABBREVIATIONS

AAP = American Academy of Pediatrics

AHA = American Heart Association

apo = apolipoprotein

ARIC = Atherosclerosis Risk in Communities

ARMY = Atherosclerosis Risk-Factors in Male Youngsters

ARYA = Atherosclerosis Risk in Young Adults

ASHFS = Australian Schools Health and Fitness Survey

ATPIII = third adult treatment panel

AUC = area under receiver-operating characteristic curve

BIF = carotid bifurcation

BMI = body mass index

CARDIA = Coronary Artery Risk Development in Young Adults

CCA = common carotid artery

CDAH = Childhood Determinants of Adult Health

CHD = coronary heart disease

CI = confidence interval

CT = computed tomography

CVD = cardiovascular disease

DISC = Dietary Intervention Study in Children

FCH = familial combined hyperlipidemia

FELIC = Fate of Early Lesions in Children

FH = familial hypercholesterolemia

FMD = flow mediated dilatation

HC = hormonal contraception

HDL = high-density lipoprotein

HR = hazard ratio

ICA = internal carotid artery

IMT = intima-media thickness

LDL = low-density lipoprotein

LRC = Lipid Research Clinics

MI = myocardial infarction

MONICA = MONItoring of trends and determinants in CArdiovascular disease

NCEP = National Cholesterol Education Program

NHANES = National Health and Nutrition Examination Survey

NHLBI = National Heart, Lung and Blood Institute

NPV = negative predictive value

OR = odds ratio

PDAY = Pathobiological Determinants of Atherosclerosis in Youth

PPV = positive predictive value

RR = relative risk

SD = standard deviation

SEP = socioeconomic position

STRIP = Special Turku Coronary Risk Factor Intervention Project for Children

TCH = total cholesterol

TG = triglycerides

US = United States

VLDL = very-low-density lipoprotein

WHO = World Health Organization

# 1. INTRODUCTION

*"Give me a child until he is seven and I will give you the man"*

St. Francis Xavier (1506 – 1552)

Although death from cardiovascular disease (CVD) has been dramatically reduced since it peaked in industrialised countries in the 1950s, it remains the single biggest killer worldwide. While favourable changes in risk factor distribution and improved treatment have been the major explanations for this decline, most of these efforts have focused on adults. Data have existed since the 1950s that demonstrated the presence of the early stages of CVD in young men and adolescent boys. If the origin of CVD is in early life, are children with one or more of the major established CVD risk factors at an increased risk of developing clinically significant disease later in life? What effect will the control of these CVD risk factors in early life have on the incidence of clinical disease later in life? Currently, there are no available data to answer these questions, but a growing level of evidence has been compiling since the 1970s to suggest that risk factor control, if implemented in early life, would indeed further reduce mortality due to CVDs. This thesis and the aims studied herein attempt to build on, and add evidence to, a component of this premise. This chapter provides an overview of the epidemiology of CVD, the pathogenesis of atherosclerosis, evidence of the childhood origin of atherosclerosis, and details essential literature that paves the way for the aims examined as part of this thesis.

## 1.1. EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

### 1.1.1 THE GLOBAL BURDEN OF CARDIOVASCULAR DISEASES

Cardiovascular diseases are the major cause of death in high- and middle-income countries and are becoming an increased burden to low-income countries.[1, 2] In 2004 the two leading causes, coronary heart disease and stroke, were responsible for 7.2 million and 5.7 million deaths respectively, accounting for 22% of total deaths (Figure 1).[1] By 2030, the World Health Organization (WHO) estimates that CVDs will remain the leading cause of death in high- and middle-income countries, and will replace respiratory infections and perinatal conditions (prematurity, low birth weight, birth asphyxia, birth trauma, and neonatal infections) as the leading cause of death in low-income countries.[1]

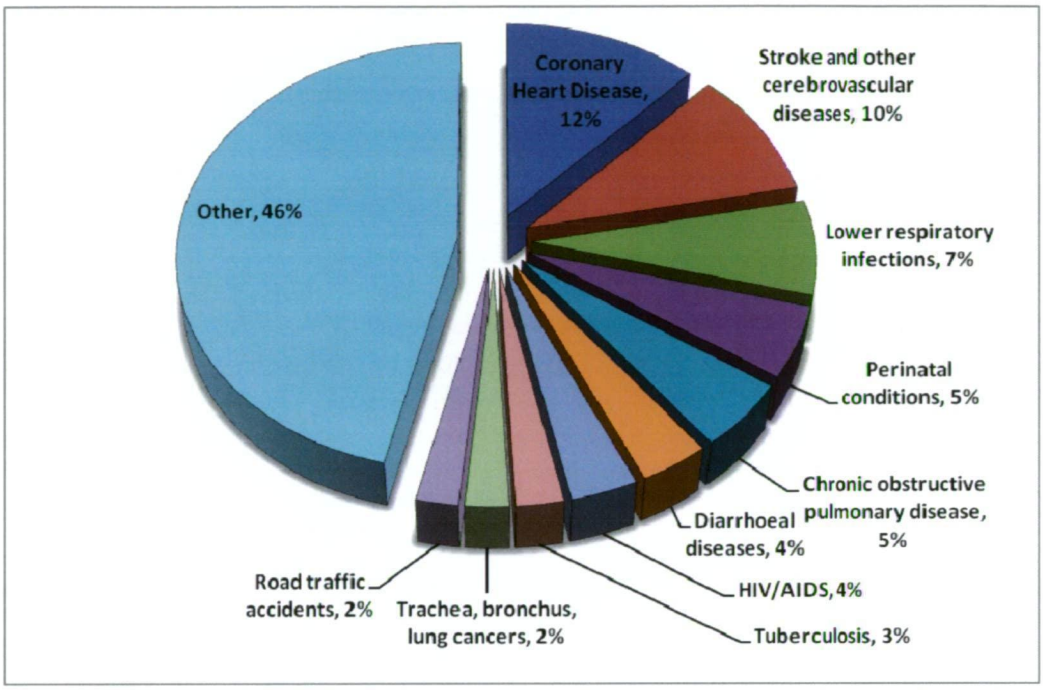


**Figure 1.** The 10 leading causes of death worldwide in 2004. Figure reproduced from data reported by the WHO[1]

### 1.1.2 SECULAR TRENDS IN CARDIOVASCULAR DISEASE

Since a peak in 1950, death rates from CVDs have declined by approximately 70% in the United States (Figure 2);[3] a trend that has been observed in most economically-developed countries.[4] The WHO's MONICA (MONItoring of trends and determinants in CArdiovascular disease) project, using standardised protocols, procedures, and quality-assurance methods, confirmed a decline in CVD mortality rates from 37 populations in 21 countries over a 10-year period.[5] Data from MONICA suggested that favourable changes in risk factors accounted for approximately 15% of the decline in CVD-event rates for females and 40% for males.[6] In the Framingham Heart Study, favourable changes in risk factors accounted for approximately half of the decline, whereas improved treatment accounted for the other half.[7] The extent and severity of atherosclerosis, a slowly progressive systemic disease that causes narrowing and hardening of large- and medium-sized arteries, also declined during this period.[8, 9]

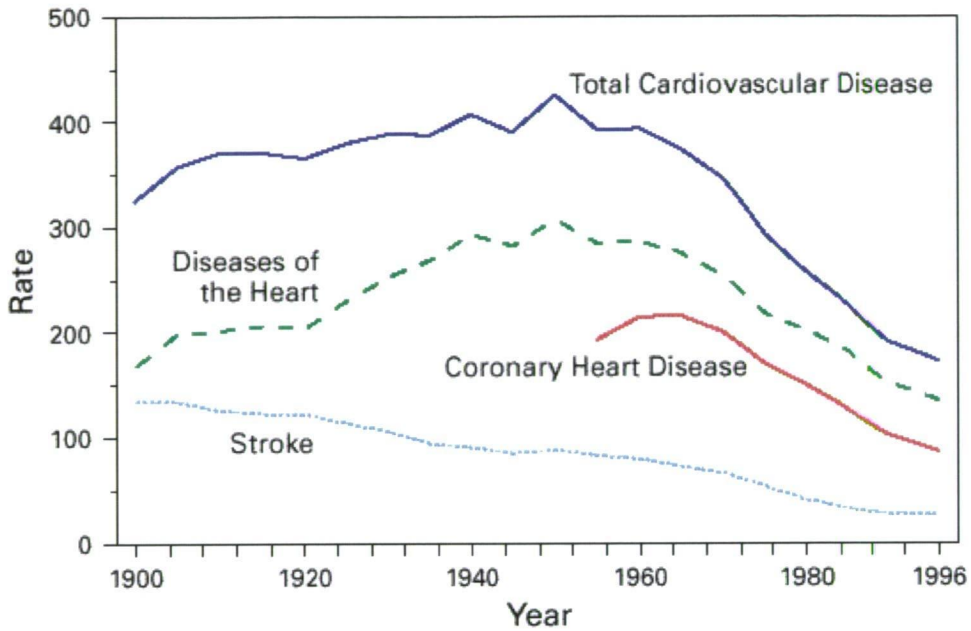


**Figure 2.** Age-adjusted death rates (per 100,000 population, standardised to 1940 US population) for total cardiovascular disease, diseases of the heart, coronary heart disease and stroke by year in the United States 1900 to 1996. From US Department of Health and Human Services, National Institutes of Health.[5, 10]

## 1.2. PATHOGENESIS OF ATHEROSCLEROSIS

The major clinical complications of atherosclerosis such as myocardial infarction, stroke, and peripheral artery disease account for approximately 50% of all deaths in economically-developed countries.[11] Atherosclerosis is characterised by the process in which deposits of fatty substances, cholesterol, cellular waste products, white blood cells, calcium and other substances build-up in the inner most layer (the intima) of an artery to form atheromas or plaques that protrude into and alter the blood flow through an artery, weaken the structural integrity of the vessel, and cause an increase in the stiffness of the vessel.[12] The coronary arteries, aorta, and cerebral arteries are the vessels most often affected. The mechanisms that lead to the initiation of atherosclerosis have not been unequivocally established. Several hypotheses have been postulated however. Of these, models placing inflammation, endothelial alterations, retained lipoproteins, and oxidation as the initiating and/or central process have received most support.[13-15] Most hypotheses share the common elements of some damage to the endothelial cells that line the arteries as well as low-density lipoprotein (LDL) cholesterol uptake and inflammation at the site, and free radical production. The causes of endothelial cell damage include elevated serum cholesterol levels (hyperlipidemia or hypercholesterolemia), free radicals caused by smoking, high blood pressure (hypertension), infection, heavy metal exposure, genetic alterations, and combinations of these and other factors.[12]

The American Heart Association (AHA) has defined six types of atherosclerotic lesions (Figure 3, Figure 4).[16, 17] The initial (type I) lesion contains sufficient levels of atherogenic lipoprotein to elicit an increase in the numbers of macrophages and cholesterol-containing macrophages (foam cells) invading the intima, resulting in adaptive thickening. Type II lesions (fatty streaks) denote the first visible sign of an arterial lesion forming. It is characterised by layers of macrophage foam cells and lipid droplets within intimal smooth muscle cells, and some evidence of droplets of extracellular fluid. Type 1 and type II lesions are often referred to as "early lesions" because they can be present in the arteries of children and adolescents and because they precede "later" or advanced lesions. Type III (intermediate) lesions, typically observed in the third decade of life, are characterised by pools of extracellular lipid in addition to all components of type II lesions. Type IV lesions (atheromas) are characterised by a dense accumulation of extracellular lipid in an extensive but well-defined region of the intima and is the first lesion considered as advanced in the AHA classification. This dense accumulation of lipid, also called the lipid core, causes severe

intimal disorganisation. During the fourth decade of life, connective tissue that forms in and around the intima as a result of a type IV lesion may lead to the formation of a thick fibrous cap that overlies either a core of lipid, calcific, or fibrotic tissue. The presence of this fibrous cap (principally comprised of smooth muscle cells and collagen) characterises the type V lesion (fibroatheroma). Type VI (complicated) lesions, the most symptomatic, are type IV or type V lesions that have become increasingly complex due to rupture (tears, fissures or ulcerations) of the surface of the lesion (fibrotic cap), hematoma or haemorrhage, thrombotic deposits, or a combination of these that results in additional damage, de-stabilisation, and thickening of the lesion accelerating the conversion from clinically silent to overt disease. The formation of a thrombus or blood clot causing acute occlusion of the artery is responsible for the most important clinical complications of atherosclerosis, myocardial infarction and stroke, which occur largely as a result of type VI lesions.

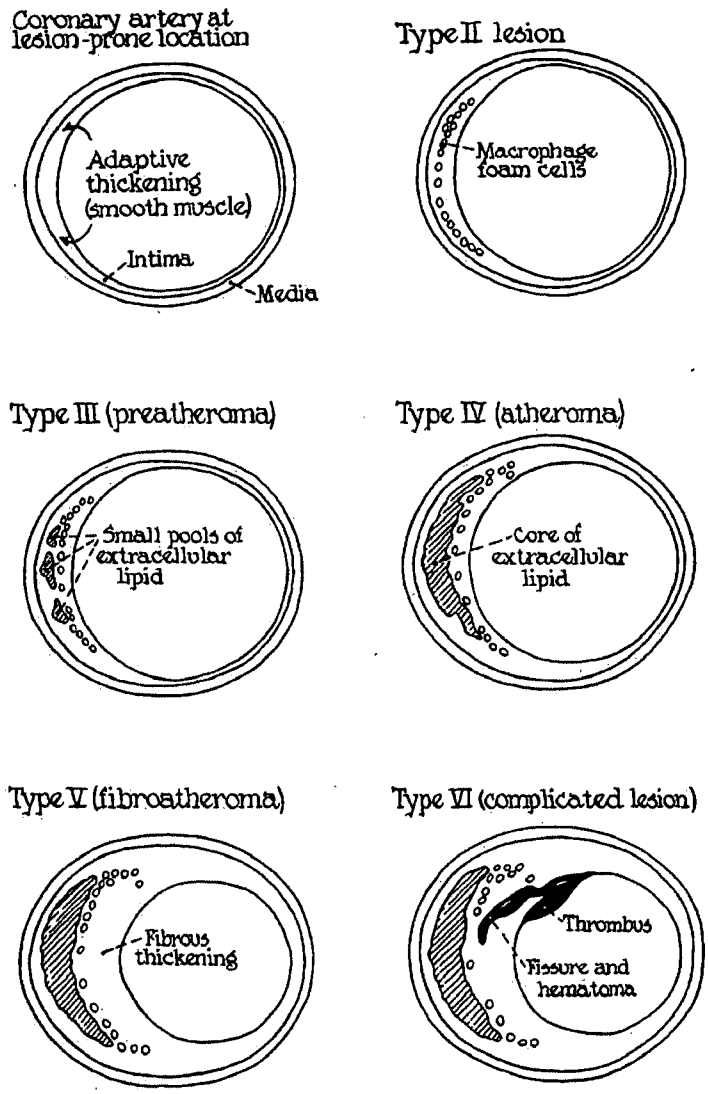


**Figure 3.** American Heart Association classification of atherosclerotic lesion types. The morphology of the intima ranges from adaptive intimal thickening (top left-hand corner) to a type VI lesion in advanced atherosclerotic disease (bottom right-hand corner). From Stary et al.[16, 17]

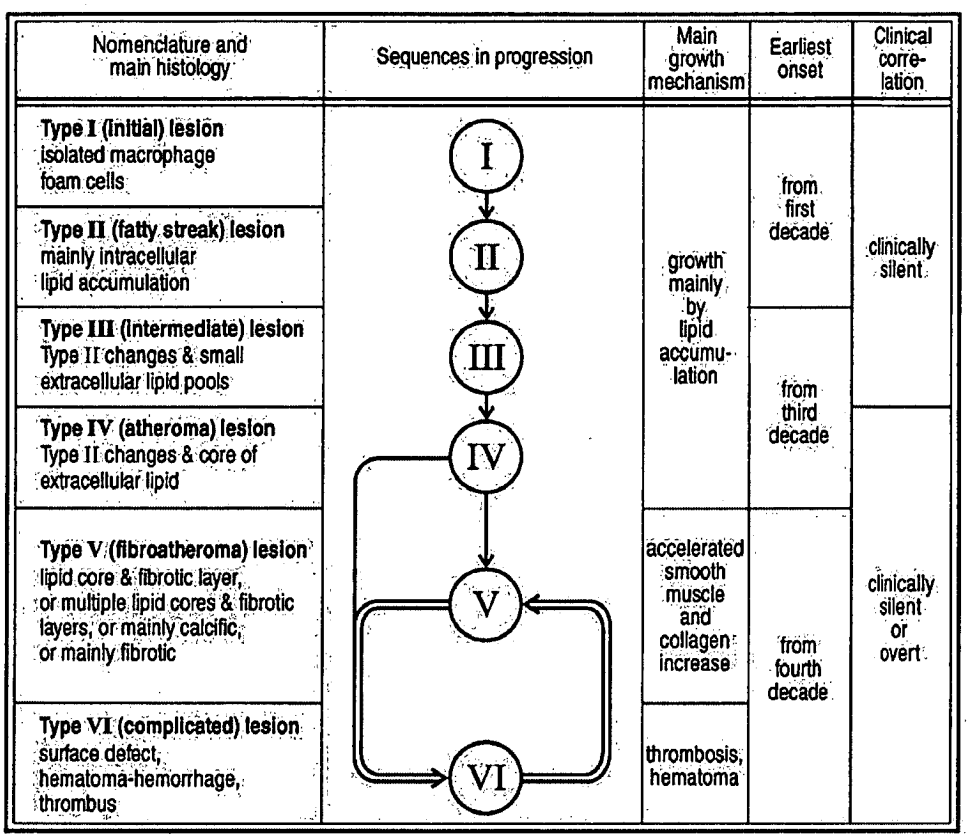


**Figure 4.** Major characteristics, possible sequence of progression, and likely onset of lesions that comprise the AHA classification. From Stary et al.[16, 17]

## 1.3. ORIGIN OF ATHEROSCLEROSIS

Although the major clinical complications of atherosclerosis do not typically present until middle or older age, the atherogenic process has been shown to begin in early life and progresses from an asymptomatic phase to a clinically manifest disease over decades. Autopsy studies on causalities of the Korean war by Enos et al,[18-20] later confirmed in the Vietnam conflict by McNamara and colleagues,[21] provided the first clinical evidence of advanced atherosclerotic lesions (grossly visible lesions that varied from mild fibrous lesions to complete coronary artery occlusion) already being present in the coronary arteries among 75% of 300 young male United States (US) soldiers (mean age of 22 years); while approximately 10% had advanced lesions that obstructed the lumen of a major vessel by 70% or more. The first evidence of atherosclerotic disease beginning in childhood was provided by Strong, McGill and colleagues[22-24] who demonstrated not only the presence of fatty streaks in the aortas of children as young as three years, but that these fatty streaks progressed to clinically significant lesions (fibrous plaques) by young adulthood (Figure 5). An extension of this work that compared pathologic findings from different geographic areas showed that fatty streaks were found uniformly at young ages, but major differences were evident in the progression to fibrous plaques between populations.[25] This difference appeared to coincide with the incidence of clinical manifestations of atherosclerosis in each geographical region. For example the rate of progression from fatty lesions to fibrous plaques occurred more rapidly in a region (New Orleans, USA) where the incidence of clinical complications was high, compared with slower progression rates in regions where incidences were lower (Guatemala and Costa Rica).

Around this time, a number of groups initiated large prospective observational studies in adults to examine the cardiovascular risk factor concept.[26-29] The cardiovascular risk factor concept proposed that there were multiple causes of atherosclerosis that, if identified, could be modified to reduce the risk of CVDs. As the effects of risk factors on adult CVD were demonstrated by studies such as the Framingham Heart Study, the Evans County study, and the Seven Countries' Study,[30-32] a number of large prospective observational studies were initiated to examine the importance of childhood cardiovascular risk factors and lifestyle to the development of atherosclerosis (Table 1).

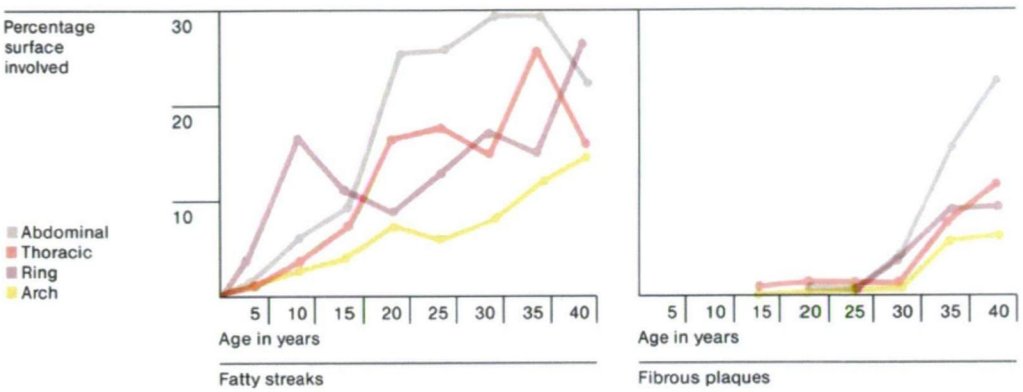


**Figure 5.** Atherosclerosis in the aorta of young men from the New Orleans area. From Berenson et al.[33]

**Table 1.** Key studies and findings with vascular (pathological or imaging) outcomes that have examined the paediatric origin of cardiovascular risk and atherosclerosis, sorted by year of commencement

| Study | Country | Year commenced | Design | Population at baseline | Vascular outcomes | Key publications |
|---|---|---|---|---|---|---|
| Muscatine Study | USA | 1971 | Longitudinal | N=4829, aged 8-18 y | Carotid calcification, carotid IMT | [34, 35] |
| Bogalusa Heart Study | USA | 1973 | Longitudinal | N=3525, aged 5-14 y at first cross-sectional examination | Autopsy (specimens included coronary arteries and aorta), arterial elasticity, carotid IMT | [36, 37] |
| The Amsterdam Growth and Health Longitudinal Study | The Netherlands | 1977 | Longitudinal | N>600, aged 13 y | Carotid IMT, arterial stiffness | [38-40] |
| Cardiovascular Risk in Young Finns Study | Finland | 1980 | Longitudinal | N=3596, aged 3-18 y | Carotid IMT, arterial elasticity, brachial FMD | [41-43] |
| Childhood Determinants of Adult Health (CDAH) Study | Australia | 1985 | Longitudinal | N=8498, aged 7-15 y | Carotid IMT, arterial elasticity, brachial FMD | [44] |
| Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study | USA | 1987 | Pathological | N=2876, aged 15-34 y | Autopsy (specimens included coronary arteries and aorta) | [45-47] |
| Northern Ireland Young Hearts Project | Northern Ireland | 1989 | Longitudinal | N=1015, aged 12-22 y | Arterial stiffness | [48, 49] |
| Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) | Finland | 1990 | Intervention | N=1062, aged 7 months | Carotid & aortic IMT, brachial FMD | [50-52] |

Abbreviations: IMT = intima-media thickness; FMD = flow-mediated dilatation

Autopsy data from the Bogalusa Heart Study[36, 53-56] and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study extended the work by Strong et al. by demonstrating that CVD risk factors measured antemortem (in the case of the Bogalusa Heart Study), and postmortem (in the case of PDAY) were associated with the presence and extent of atherosclerosis in the aortic and coronary arteries of young persons (ranging in age from 2-39 years), and provided the first evidence that risk factor control beginning in young persons may delay the development of atherosclerosis. The advancement in novel non-invasive techniques throughout the 1990s allowed functional and structural markers of early (pre-clinical) atherosclerosis to be measured in young, apparently healthy populations.[57-59] Such measures included the ultrasonic assessment of carotid artery intima and media thickness (IMT), arterial endothelial function using flow-mediated dilatation (FMD), and arterial elastic properties, as well as computed tomography to measure coronary artery calcification (see Table 1 for a summary of these methods and Appendix 1 for a more detailed overview of each technique). Once the reproducibility and prognostic value of these measures was established, they provided an intermediate end-point that could be used to assess the effects of risk factors and risk-factor interventions long before clinical manifestations of CVD would present. Utilising non-invasive imaging technologies, longitudinal studies were able to provide data directly linking childhood risk factors with preclinical measures of atherosclerosis in young adulthood.[34, 35, 37, 41, 43] Landmark papers from the Muscatine Study (1996, 2001),[34, 35] Bogalusa Heart Study (2003),[37] and Cardiovascular Risk in Young Finns Study (2003, 2004, 2005)[41-43] together have accumulated over 900 citations in the scientific literature since they were first published, clearly demonstrating the importance of these data.

Two other extremely important observations from both pathological and imaging studies of atherosclerosis have been that the extent of lesions or indicators of atherosclerosis increases as the number of childhood risk factors increases, suggesting that risk factors measured in childhood appear to be stronger predictors of future atherosclerosis than contemporous measurements obtained at the time autopsy or imaging was performed. Together, these findings provide a strong rationale for both the identification of children at high-risk and for efforts to prevent the development of CVD risk factors in early life. The following section provides an overview of the major paediatric CVD risk factors that have been linked with atherosclerosis, before focusing on the importance of blood lipid and lipoprotein levels in childhood and adolescence in the development of atherosclerosis.

**Table 2.** Most common non-invasive imaging techniques used to assess preclinical atherosclerosis in studies of children, adolescents and young adults

| | Arterial Phenotype | | | |
|---|---|---|---|---|
| | Intima-media thickness (structural)[60, 61] | Flow-mediated dilatation (functional)[62, 63] | Arterial stiffness (mechanical)[64, 65] | Calcification (structural) |
| Premise | -Diffuse intimal thickening occurs early in atherosclerosis, prior to lesion development. | -The endothelium regulates vascular homeostasis and has antithrombotic and antiproliferative properties. Nitric oxide in the endothelium plays a major role in both actions.<br>-Alteration in endothelial function precedes structural atherosclerotic changes. | -Proximal large arteries have high elastic properties that are a result of the high elastin to collagen ratio in their walls. The gradual loss of elastic properties of these variables with aging is a result of progressive elastic fibre degeneration.<br>-Decreased elasticity is thought to represent an early risk marker for CVD. | -Coronary artery calcification is part of the pathogenesis of atherosclerosis.<br>-It occurs almost exclusively in atherosclerotic arteries.<br>-Is absent in the normal vessel wall. |
| Technology | B-mode ultrasound | B-mode ultrasound | B-mode ultrasound, Doppler | Computed tomography (CT) |
| Limitations | -Not able to differentiate between intima and media, so both are measured.<br>-Non-standardised protocols.<br>-Imperfect correlation with coronary atherosclerosis. | -Requires highly-skilled technicians.<br>-High normal physiological variability.<br>-Degree of dilatation can be effected by: recent smoking, recent food/beverage intake; recent exercise, circadian pattern; viral illness; and phase of the menstrual cycle for females.<br>-Imperfect correlation to coronary circulation.<br>-Vasodilatory response is related to vessel size. | -Large number of different protocols and equipment used, each with strengths and limitations:<br><br><u>Pulse Wave Velocity</u><br>-Does not provide information on arterial geometry.<br>-Inaccuracy of distance measurement.<br><br><u>Central pulse-waves</u><br>-Indirect measure of arterial stiffness.<br><br><u>Change in vessel diameter to distending pressure</u><br>-Requires skilled technician<br>-Requires measurement of central pulse pressure. | -Radiation exposure<br>-High rates of zero scores before 5th decade.<br>-'Noise' can be mistaken for calcium on CT images leading to higher scores.<br>-Cost<br>-More recent technologies have high reproducibility, but earlier technologies had poor short-term reproducibility.[66]<br>-Vulnerable plaque may not exhibit significant calcification. |
| Predictive value* | ++ | + | + | +++ |
| Simplicity* | ++ | + | ++/+++/+ | + |
| Reproducibility* | +++ | + | ++/++/+ | ++ |
| Safety* | +++ | +++ | +++/+++/+++ | + (radiation) |
| Low cost* | ++ | ++ | +++/+++/++ | + |

* Ratings based on review articles of these measures.[60, 63-67] + = fair, ++ = good, +++ = excellent.

## 1.4. RISK FACTORS FOR ATHEROSCLEROSIS IN CHILDREN AND YOUNG ADULTS

The value of a number of non-modifiable and modifiable risk factors for CVD risk prediction (including increasing age, male sex, race, heredity, high-risk blood lipid levels, high blood pressure, smoking, overweight and obesity, diabetes and physical inactivity) in adult studies have also been shown to be important in the development and progression of preclinical atherosclerosis in young persons.

### 1.4.1 NON-MODIFIABLE RISK FACTORS

#### 1.4.1.1 Increasing age

In the PDAY study, increasing age was found to be significantly associated with the extent of fatty streaks and raised lesions, and a two to three fold increase in the prevalence of AHA type IV and V lesions in the coronary arteries of deceased persons.[45, 46] Data from Holman et al.[22] presented in Figure 5 demonstrated that the extent of fatty streaks in the aorta of young men increased with age. In a cross-sectional study by Ishizu et al.[68] increasing age was shown to be directly associated with mean and maximum IMT in children aged 5 to 14 years (mean IMT, $\beta$ = 0.009 mm/year; max IMT $\beta$ = 0.010 mm/year). Age was related with significant increases in composite (multiple sites of measurement) IMT in Caucasian males (0.012 mm/year), Caucasian females (0.005 mm/year), and African-American females (0.008 mm/year) in the Bogalusa Heart Study.[69] Data from the Cardiovascular Risk in Young Finns study showed that age was directly associated with carotid IMT (0.06 mm/year) and inversely associated with carotid artery compliance (-0.04 %/10 mmHg/year) even after adjustment for other risk factors.[70] While data from the Coronary Artery Risk Development in Young Adults (CARDIA) study observed a greater prevalence of coronary artery calcification in older participants compared with younger participants (13.3 vs. 5.5%).[71]

#### 1.4.1.2 Male sex

Using a very high-resolution ultrasound technique that could differentiate intima and media, Osika et al recently demonstrated that male students from two schools in Gothenburg had significantly larger intima (0.057 vs. 0.054 mm), media (0.176 vs. 0.153 mm), and IMT measurements (0.232 vs. 0.207 mm) in the radial artery and significantly larger media (0.160

vs. 0.149 mm), and IMT measurements (0.222 vs. 0.209 mm) in the dorsal pedal arteries compared with female students.[72] In the PDAY study, females had approximately half the extent of raised lesions in the right coronary artery and approximately half the prevalence of advanced lesions in the left anterior descending coronary artery compared with their age-matched male counterparts.[46, 73] The CARDIA study found young adult males to have a greater prevalence of coronary calcium than females (15.0 vs. 5.1%).[71] Data from the Bogalusa Heart Study also suggested that lesion development was delayed in females compared with males,[54] while males had increased carotid IMT (0.757 vs. 0.719 mm)[37] and greater age-related changes in IMT compared with females.[69] These studies however, did not examine if these differences remained after adjustment for risk factors. In the Cardiovascular Risk in Young Finns study, male sex was associated with significantly increased carotid IMT (0.59 vs. 0.57 mm), decreased carotid compliance (2.00 vs. 2.31 %/10 mmHg), and decreased FMD (6.95 vs. 8.83 %).[70] These differences became non-significant after adjustment for risk factor levels in the case of IMT and carotid compliance, and reversed after adjustment for baseline brachial diameter in the case of FMD; suggesting that sex differences in markers of atherosclerosis were mostly explained by differences in risk factors and vessel size.[70]

#### 1.4.1.3 Race

Studies in the United States have shown that while African-Americans have higher levels of CVD risk factors,[74] it is Caucasians that have the highest incidence of CVD.[75] Pathological studies in youth and young adults have found more extensive fatty lesions in the arteries of African-Americans compared with Caucasians, seemingly reflecting increased risk factor load, but similar amounts of raised lesions have been observed. In the PDAY study, minor differences were observed between Caucasian and African-Americans in extent and qualities of lesions.[73] In the Bogalusa Heart Study, African-Americans had a greater extent of fatty lesions in the aorta and Caucasian males a greater prevalence of fibrous plaques in the coronary artery,[76] Although autopsied decedents may not be representative of the living population, this race difference has been shown in young adults in the Bogalusa study. For example, African-American males and females had greater levels of carotid IMT compared with Caucasian males (0.770 vs. 0.751 mm) and females (0.753 vs. 0.705 mm).[37] In the CARDIA study no significant race differences were reported initially,[74] but more recently, Caucasian males had a greater prevalence of coronary artery calcium than African-American males (17.6 vs. 11.3%).[71] In the Bogalusa Heart Study, Caucasian males had greater age-

related IMT changes over a five year period than African-American males (0.009 vs. -0.001 mm/year).[69] While studies are continuing to examine racial differences in atherosclerosis, it is possible that Caucasians experience more rapid progression to advanced lesions than do African-Americans, possibly in early to middle adulthood.

#### 1.4.1.4 Heredity

Children who are first-degree relatives of persons with premature CVD have been shown to have reduced endothelial function and increased IMT. Gaeta et al.[77] found that 40 healthy adolescent offspring of parents who had premature myocardial infarction had lower brachial FMD (5.7 vs. 10.2%) and increased carotid IMT (0.49 vs. 0.44 mm) compared with 40 age- and sex-matched controls. These differences between groups remained significant after adjustment for risk factors,[77] suggesting that vascular changes associated with positive family history are independent of risk factor levels. Cuomo et al. also showed that children, adolescents, and young adults with a history of premature parental myocardial infarction had increased carotid IMT compared with age- and sex-matched controls (age 5-18 years: 0.45 vs. 0.40 mm; age 19-30 years: 0.48 vs. 0.45 mm).[78] As in the Gaeta study, differences in carotid IMT between the two groups remained after adjustment for other risk factors.[78] A study by Magadle et al. found that young adults with family history of premature myocardial infarction had increased carotid IMT (0.48 vs. 0.43 mm) compared with age-, sex-, and risk factor-matched controls.[79] Sabri et al.[80] demonstrated that child offspring of those with premature myocardial infarction had significantly increased carotid IMT (0.29 vs. 0.22 mm) and left ventricular mass (37.8 vs. 36.2 $g/m^{2.7}$) compared with age- and sex-matched controls who did not have positive family history. While the evidence for increased atherosclerosis in the offspring of persons who suffered premature myocardial infarction appears compelling, data on the vasculature of offspring of parents who suffered premature stroke is sparse. To the best of the author's knowledge, the only study by Varda et al.[81] to examine this issue found that carotid IMT at multiple sites was not significantly different in children of parents with or without premature stroke. In both the Bogalusa Heart Study[82] and the Cardiovascular Risk in Young Finns Study,[83] the adverse effects of metabolic risk factors were amplified in those with positive family history of CVD. While the Bogalusa Heart Study showed that family history of CVD (parental, any age) predicted five-year carotid IMT progression in young men ($\beta = 0.014$ mm).[84]

## 1.4.2 MODIFIABLE RISK FACTORS

### 1.4.2.1 Hypertension

A number of cross-sectional and case-control studies have shown that children and adolescents with primary (essential) hypertension have significantly greater values of carotid and femoral IMT and reduced arterial elasticity compared with those without hypertension.[85-88] In the PDAY study, hypertensive (defined as mean arterial pressure ≥110 mm/Hg) males had approximately twice the prevalence of raised lesions in the right coronary artery compared with their normotensive peers.[89] Hypertension was also associated with larger diameters of the right coronary and left anterior-descending arteries and with the prevalence of AHA grade IV and V lesions.[90] These associations remained after adjustment for other CVD risk factors including body mass index (BMI), smoking, and lipoprotein levels.[89, 90] In the Bogalusa Heart Study,[36] systolic blood pressure was positively correlated with the extent of fatty streaks in the aorta ($r = 0.31$) and with fatty streaks and fibrous plaques in the coronary arteries ($r = 0.47$, and $r = 0.41$ respectively).

In living participants, systolic blood pressure was a significant independent predictor of five-year internal carotid IMT progression in young men of the Bogalusa Heart Study ($\beta$ = 0.001 mm per 1mm/Hg increase),[84] while childhood and cumulative (measures collected at multiple time-points) systolic blood pressure was directly associated with carotid IMT ($r$ = 0.103, and $r$ = 0.165 respectively)[37] and arterial stiffness ($r$ = 0.11, and $r$ = 0.32 respectively).[91] A direct and significant relationship between a single measure of systolic blood pressure taken in adolescence and arterial stiffness in young adulthood was however, not found in the Atherosclerosis Risk in Young Adults (ARYA) study. But measurement error in the use of a single baseline measurement of blood pressure may have partly explained the lack of association.[92] In the Muscatine study, systolic and diastolic blood pressures measured in early adult life but not childhood were associated with increased odds (ranging from 3.2 to 6.4) of coronary artery calcium as measured by electron-beam computed tomography.[34] Systolic and diastolic blood pressures in childhood were directly associated with carotid IMT in Muscatine women, but these associations were attenuated after adjustment for current risk factors.[35] In the Cardiovascular Risk in Young Finns study, childhood systolic blood pressure was positively associated with adult carotid IMT[41] ($\beta$ = 0.013 mm per 1 standard deviation [SD] increase) and inversely associated with adult

measures of carotid elasticity[43] (β = -0.107 %/10 mmHg per 1SD increase) and brachial FMD in male adults (β = -0.049% per 1SD increase).[93] Importantly, the prospective association observed between blood pressure and carotid IMT and brachial endothelial function was stronger than the cross-sectional association.[41, 93] In the 10-year follow-up of the CARDIA study, baseline and current systolic blood pressure levels were associated with increased odds ratios (OR = 1.3, and OR = 1.3 per 10 mm/Hg increase respectively) of presence of coronary calcium, but both associations were attenuated and no longer statistically significant after adjustment for other CVD risk factors.[74] At the 15-year follow-up, baseline, year 15, and average (from years 0, 2, 5, 7, 10, and 15) systolic blood pressure levels were significantly associated with increased odds of coronary calcium (baseline OR = 1.3, 15-year OR = 1.3, average years 0 to 15 OR = 1.6 per 10 mm/Hg increase) after adjustment for other risk factors (LDL cholesterol, high-density lipoprotein [HDL] cholesterol, cigarette smoking, BMI, and glucose).[71] Also in this study, systolic blood pressure was the only risk factor examined where 15-year change in levels predicted coronary calcium levels more strongly than the baseline level (change OR = 1.20 vs. baseline OR = 1.16 per 10 mm/Hg).[71]

### 1.4.2.2 Smoking

Autopsy studies in the young demonstrate robust associations between exposure to cigarette smoking and extent of atherosclerotic lesions. In the PDAY study, smoking (defined as having a serum thiocyanate level of ≥90 μmol/L) was associated with more extensive fatty streaks and raised lesions in the abdominal aorta[94] even in those with favourable lipoprotein profiles,[73] and a higher microscopic grade of atherosclerosis in the left anterior descending coronary artery,[95] but not the right coronary artery.[73] In autopsy studies from the Bogalusa Heart Study,[36] the percentage of the intimal surface involved with fatty streaks in the coronary arteries was significantly higher in smokers compared with non-smokers (8.27 vs. 2.89 %), as was the percentage of fibrous plaque in the aorta (1.22 vs. 0.12 %). The prevalence of fatty streaks and fibrous plaques in the aorta and coronary arteries were also increased in smokers compared with non-smokers but the association was non-significant, probably owing to low statistical power.[36]

In a series of case-control studies, Celermajer and colleagues in the early 1990s were the first to provide landmark evidence linking exposure to cigarette smoking and exposure to environmental tobacco smoke (including side-stream smoke from a burning cigarette and exhaled smoke) in living young persons to early signs of atherosclerosis.[96-98] Never smokers

had significantly higher endothelium-dependent FMD compared with current smokers (10.0 vs. 4.0 %) in multivariable analyses adjusted for age, sex, cholesterol and vessel size, while lifetime dose smoked (pack years) was inversely associated with FMD (r = -0.33). Endothelium-dependent FMD was also higher in former-smokers compared with current smokers (5.1 vs. 4.0%) in multivariable analyses.[97] A second study from the group showed that endothelium-dependent FMD was reduced not only in smokers but also in those exposed regularly to environmental (passive) tobacco smoke (controls, FMD = 8.2%; smokers, FMD = 4.4%, passive smokers, FMD = 3.1%).[98] In passive smokers, the intensity of exposure to environmental tobacco smoke was inversely related with FMD (r = -0.67). These data showed that cigarette smoking and passive smoking was associated in a dose-dependent, but potentially reversible, impairment in endothelial function.

Cross-sectional data from the Atherosclerosis Risk-Factors in Male Youngsters (ARMY) study showed that adolescent smoking was significantly associated with prevalence of high IMT in a linear fashion (non-smokers, OR = 1.0; 1 to 9 cigarettes per day, OR = 2.7; 10-19 cigarettes per day, OR = 3.6; ≥20 cigarettes per day, OR = 5.0).[99] A sub-analysis of the Special Turku Coronary Risk Factor Intervention Project (STRIP) demonstrated that the association between tobacco smoke exposure and decreased endothelium-dependent flow-mediated dilation was dose dependent (FMD for the noncotinine group = 9.1%, the low-cotinine group = 8.6%, and the top-decile cotinine group = 7.7%), suggesting that even modest exposure to tobacco smoke in childhood may be hazardous.[100] The Cardiovascular Risk in Young Finns study showed that carotid IMT was significantly associated both with childhood smoking[41] and with various definitions of young adult smoking (ever, current, cigarettes per day, pack-years of smoking).[42] In the CARDIA study, baseline cigarettes per day were associated with increased odds (OR = 1.4 per 10 cigarettes/day) of coronary calcium at year 15 but not change in cigarettes smoked per day between baseline and year 15.[71] In the Bogalusa Heart study, current smoking was the most consistent predictor of composite IMT progression in males (β = 0.017 mm, compared with current non-smokers) and females (β = 0.011 mm, compared with current non-smokers).[84]

### 1.4.2.3 Overweight and obesity

The role of excess adiposity (overweight and obesity) in youth is one of few risk factors that have been linked with incident cardiovascular events in adulthood. Findings to date however have been equivocal. For example, data from 11,000 persons born in Aberdeen, Scotland,

found that BMI measured at age 5 years was not associated with future risk of coronary heart disease.[101] Another study that combined data on more than 23,000 participants from three historical cohorts (Boyd Orr, Christ's Hospital, and Glasgow Alumni cohort) that collected height and weight measures in childhood and adolescence found that overweight and obese children were not at significantly increased risk of ischemic heart disease (hazard ratio, HR = 1.34, 95% confidence interval, CI = 0.95 to 1.91) or stroke (HR = 0.94, 95%CI = 0.82 to 1.08).[102] Both these studies were likely underpowered given there were only few children at the extreme (overweight or obese) end of the distribution, which is in contrast to contemporary children. The best evidence to date on the effect of childhood adiposity on coronary heart disease risk in adulthood was provided in a landmark study by Baker et al.[103] Using data from almost 300,000 participants, the authors were able to demonstrate that the risk of any coronary event in adulthood was positively associated with BMI in boys (9-20% increase per 1-unit increase in BMI z-score) and girls (4-15% increase per 1-unit increase in BMI z-score). The data showed a strikingly linear trend for increased risk across the entire BMI distribution (Figure 6), suggesting that even a small increase in childhood adiposity may increase risk in adulthood.

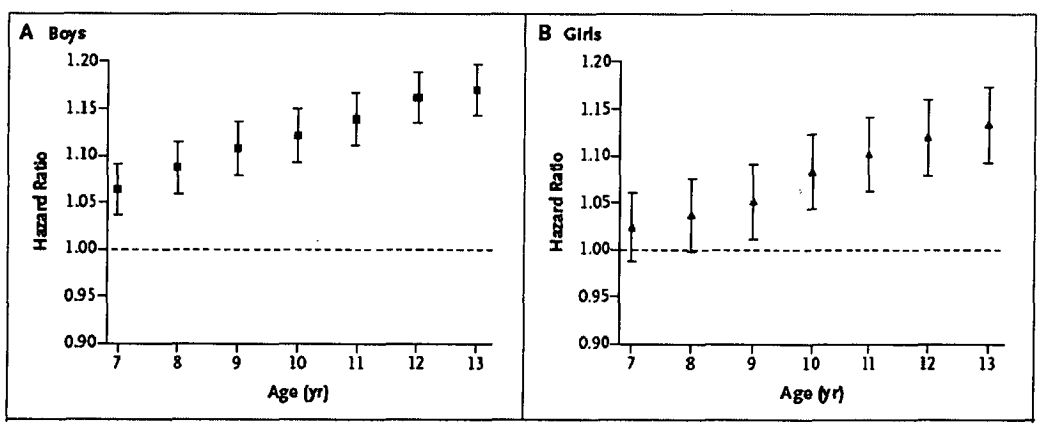


**Figure 6.** BMI in childhood and risk of any coronary heart disease event in adulthood. From Baker et al.[103]

Evidence from pathological and imaging studies has shown much more consistent trends regarding childhood adiposity and atherosclerosis. In age- and/or sex-matched case-control studies, excess BMI in youth was shown to be associated with endothelial dysfunction,[104-107] increased carotid IMT,[105, 106, 108] and increased arterial stiffness.[104, 107, 108]

The study by Aggoun and colleagues demonstrated that impaired endothelial and smooth muscle function as well as arterial stiffness develops before puberty in obese children. In the Bogalusa and PDAY studies, BMI[36] and a thick panniculus adiposus[46, 109] (a marker of visceral fat) were strong independent (of other risk factors) predictors of the extent of atherosclerosis in deceased young persons.

In longitudinal analyses, childhood adiposity has also been shown to be a significant predictor of adult vascular measures. In the Amsterdam Growth and Health Longitudinal Study, childhood BMI and sum of skin folds were significantly associated with carotid IMT (BMI, r = 0.19; sum of skin folds, r = 0.25) independent of current risk factors (pulse pressure, blood lipids, glycated haemoglobin, resting heart rate and cardiorespiratory fitness), but the authors did not examine whether the effect of childhood adiposity was independent of adult adiposity.[40] Also, adolescent truncal subcutaneous fat was inversely associated with carotid artery compliance (r = -0.18) after adjustment for current risk factors (as above). In the Cardiovascular Risk in Young Finns Study, adolescent BMI was a significant predictor of adult carotid IMT ($\beta$ = 0.01 mm for a 1 standard deviation change) in a multivariable model that also contained adolescent measures of LDL cholesterol, systolic blood pressure and smoking.[41] Additional analyses of these data showed that adult IMT values were comparable in subjects who had been overweight or obese in both childhood and adulthood and in subjects who became obese in adulthood, 0.642 mm versus 0.634 mm; whereas IMT values were lower and comparable in subjects who were not overweight or obese at both time-points and those who had been overweight or obese in youth but were not in adulthood, 0.610 mm versus 0.627 mm.[110] The authors concluded that while childhood adiposity predicts adult IMT, the effect is explained by significant tracking of adiposity from childhood to adulthood and is not independent of adult adiposity. Data from the ARYA study also found that while adolescent levels of BMI were significantly associated with carotid IMT ($\beta$ = 0.002 mm per standard deviation increase in adolescent BMI), the association was attenuated after adjustment for adult BMI ($\beta$ = 0.001 mm).[111] These findings however were in contrast to those from the Bogalusa Heart Study. For example, Li et al. found that childhood but not adulthood BMI significantly increased the odds for high carotid IMT (child OR = 1.25, adult OR = 1.09),[37] while Freedman et al. showed that the association between childhood BMI and adult IMT persisted but was reduced after adjusting for adult BMI,[112] suggesting an independent effect of childhood BMI on adult IMT. In the Muscatine study, children in the upper decile of weight were at a three-fold increase in odds of having calcium in the coronary

arteries compared with children in the lower nine deciles.[34] In the CARDIA study, young adulthood BMI ≥25 kg/m$^2$ but not change in BMI between baseline and follow-up was associated with 50% increased odds of having coronary calcium at year 15 in a multivariable model that included age, race, sex, blood lipids, blood pressure, glucose, and smoking.[71]

### 1.4.2.4 Diabetes and associated abnormalities

There is evidence that diabetes mellitus and metabolic-related risk factors in youth are associated with atherosclerosis. For example, publications from Jarvisalo,[113-115] Singh[116] Krantz,[117] Atabek,[118] Dalla Pozza,[119] and Schwab,[120] have found that children and adolescents with type 1 diabetes had impaired endothelial function,[115, 116] increased arterial stiffness,[118] and increased arterial IMT[113, 114, 117-120] compared with matched non-diabetic control children and adolescents. In these studies, the degree of endothelial impairment and increased IMT in those with type 1 diabetes appeared to be a result of duration of diabetes, presence of diabetes complications, blood pressure (systolic and diastolic) or LDL cholesterol levels.[113, 116, 117, 120] [119] Currently, there are no published data available that examine preclinical atherosclerosis in those children with type 2 diabetes. In the PDAY study, autopsied youth with hyperglycaemia (measured using level of glycohemoglobin in the blood) had increased odds of lesions in the coronary arteries (OR = 2.6) and the abdominal aorta (OR = 2.3).[47] Insulin resistance in adolescents has been cross-sectionally linked with brachial distensibility;[121] while data from the Cardiovascular Risk in Young Finns Study suggested a longitudinal inverse association of childhood insulin levels with carotid arterial elasticity in adulthood (r = -0.17), but did not remain a significant predictor in the final multivariable model that included sum of skin folds.[43]

Presence of the metabolic syndrome, a cluster of cardiometabolic risk factors including obesity, hypertension, dyslipidemia, and impaired glucose tolerance,[122] is associated with increased risk of developing type 2 diabetes[123] and CVD[124-126] in adults. Currently, there is no consensus definition for what constitutes metabolic syndrome in children and adolescents,[127] although a number have been proposed.[128-132] Moreover, no published data have examined the ability of these different paediatric definitions to predict markers of atherosclerosis in adulthood. A recent cross-sectional study on 264 overweight or obese adolescents found that the metabolic syndrome definition indicated by Weiss and Viner significantly predicted carotid IMT, but other definitions were not associated with carotid IMT.[133] A report by Iannuzzi et al.[134] found obese children with metabolic syndrome to have

increased carotid artery stiffness compared with obese children who did not have metabolic syndrome, which persisted after adjustment for age, sex, and C-reactive protein. There has been a recent attempt by Morrison and colleagues to examine in the Princeton Lipid Research Clinics Follow-up Study the relation between metabolic syndrome in children and adolescents aged 5 to 19 years with CVD 25 years later.[135] In the 771 participants followed into adulthood, there were only 17 cases of participant-reported CVD at the follow-up examination. Using a definition that had been previously used in National Health and Nutrition Examination Survey (NHANES) data, the authors found that those with paediatric metabolic syndrome had 15 times the odds (95% CI = 5-45) of having CVD in adulthood compared with those children who were not classified as having metabolic syndrome.[135] Clear limitations of this study were the low case numbers and the non-use of hospital records to ascertain case information. Although it is clear the current available evidence on the association between diabetes and related metabolic abnormalities in childhood with atherosclerosis is at an early stage, the available data do suggest that diabetes and the metabolic syndrome in childhood may indeed be risk factors for atherogenesis. Given the close relationship between obesity, insulin resistance, metabolic syndrome, and type 2 diabetes and childhood trends of increasing prevalence for each of these factors since the 1980s, more robust data linking them with atherosclerosis is likely forthcoming.

### 1.4.2.5 Physical inactivity

There is good epidemiological and clinical evidence in adults that suggest physical inactivity and poor cardiorespiratory fitness are major risk factors for atherosclerosis, with the increased risk similar to that seen for conventional CVD risk factors of hypercholesterolemia, hypertension, and blood pressure.[136] Data from prospective cohort studies in older adults have demonstrated that low cardiorespiratory fitness, and low physical activity[137] as well as declining cardiorespiratory fitness[138] and declining physical activity[139] have been associated with increased risk for clinical CVD events; while clinical trials have provided data on the antiatherogenic effect of improving cardiorespiratory fitness and regular physical activity.[140] Despite comprehensive evidence in adulthood, comparatively limited data exist that have examined childhood exposure to physical inactivity or low fitness with atherosclerosis (even when compared to the available data on other childhood risk factors). The Bogalusa and PDAY studies did not collect physical activity or fitness data, therefore no data from these studies were available to link with atherosclerotic lesions. The Muscatine and Young Finns

studies have collected childhood measures of fitness and physical activity but to date, no publications have reported their association with preclinical markers of atherosclerosis. In the available literature, the evidence does suggest that physical inactivity and low cardiorespiratory fitness in childhood and adolescence are associated with markers of atherosclerosis.

Short-term clinical trials in overweight and obese youth have shown that aerobic exercise interventions improved cardiorespiratory fitness,[141, 142] increased endothelial function[142-146] and regressed carotid intima-media thickness.[146] The study by Kelly et al.[142] showed that improvements in endothelial function after 8 weeks of aerobic exercise training in overweight youth occurred without a loss of body weight or adiposity, suggesting a direct benefit of exercise on arterial function. Only two studies have examined the cross-sectional association between habitual physical activity and endothelial function. A study by Abbot and colleagues found that increasing habitual physical activity levels was the strongest multivariable determinant of brachial artery FMD ($r = 0.39$) in 47 children.[147] A recent publication from the STRIP cohort by Pahkala et al.[52] showed that leisure-time physical activity was directly associated with brachial FMD in 13 year old boys ($\beta = 0.026\%$ per MET h/wk) but not 13 year old girls. The association in boys remained significant after further adjustments for BMI, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, and C-reactive protein ($\beta = 0.028\%$ per MET h/wk).[52] No studies have examined the cross-sectional association of cardiorespiratory fitness and markers of atherosclerosis in childhood or adolescence. Cross-sectional data from the Northern Ireland Young Hearts Project found cardiorespiratory fitness and sports-related physical activity (but not leisure- or work-related physical activity) to be inversely associated with arterial stiffness in 405 young adults aged 20 to 25 years independent of age, sex, height, weight, mean arterial pressure, smoking behaviour, alcohol consumption, fat intake, and sum of four skin folds.[48]

Longitudinal evidence linking childhood physical activity or fitness to adult markers of atherosclerosis is also limited. In the Amsterdam Growth and Health Longitudinal Study, VO2max measured in adolescence (13-16 years) was independently (model adjusted for height, weight, sum of four skin folds, systolic and diastolic blood pressures, total and HDL cholesterol, resting heart rate and smoking status) associated with lower carotid IMT in males at age 36 years.[38] VO2max was not associated with carotid IMT in females nor carotid stiffness in both sexes.[38] A second publication by this group found that changes in VO2max levels between childhood and adulthood was inversely associated with arterial stiffness in the

carotid and femoral arteries but was not associated with carotid IMT.[39] Currently, no data are available that examine the association between childhood physical activity and adult markers of atherosclerosis. Given the extent of physical activity and fitness data available at baseline examination[148, 149] and the collection of imaging data at follow-up,[44, 150] it is likely the Childhood Determinants of Adult Health Study will be the first to comprehensively examine these associations. The limited evidence that exists however suggests that physical activity and fitness levels in youth may have a direct impact (independent of adiposity) on the development of atherosclerosis.

### 1.4.2.6 Multiple risk factors

Evidence from epidemiological studies shows that multiple risk factors increase the probability of cardiovascular events, since CVD risk factors tend to reinforce each other in their influence on morbidity and mortality.[151] The importance of multiple risk factors in youth has been documented in several studies. The Bogalusa Heart Study demonstrated that the degree of fatty streaks and fibrous plaques accelerate in the aorta and coronary arteries of autopsied individuals with increasing number of risk factors (including BMI, systolic blood pressure, triglycerides, and LDL cholesterol, Figure 7).[36] Longitudinal analyses of free-living participants demonstrated a similar association with the outcomes of carotid IMT[152-154] and decreased carotid artery elasticity[155] across sex- and race-strata. Data from the Cardiovascular Risk in Young Finns Study demonstrated that in every category of adults with 0, 1, or 2 or more current risk factors, there was a significant increasing trend in carotid IMT values according to the number of childhood risk factors (Figure 8).[41] Additional data from this group showed that the number of risk factors was correlated with IMT in subjects with impaired and intermediate endothelial function, whereas there was no significant correlation between the number of risk factors and IMT in subjects with enhanced endothelial function.[42] These data suggest that endothelial function in adulthood may offer some protection for arteries against the development of atherosclerosis in response to early-life exposure to risk factors. Another study from the Young Finns cohort showed that carotid artery elasticity decreased as more childhood risk factors were present.[43]

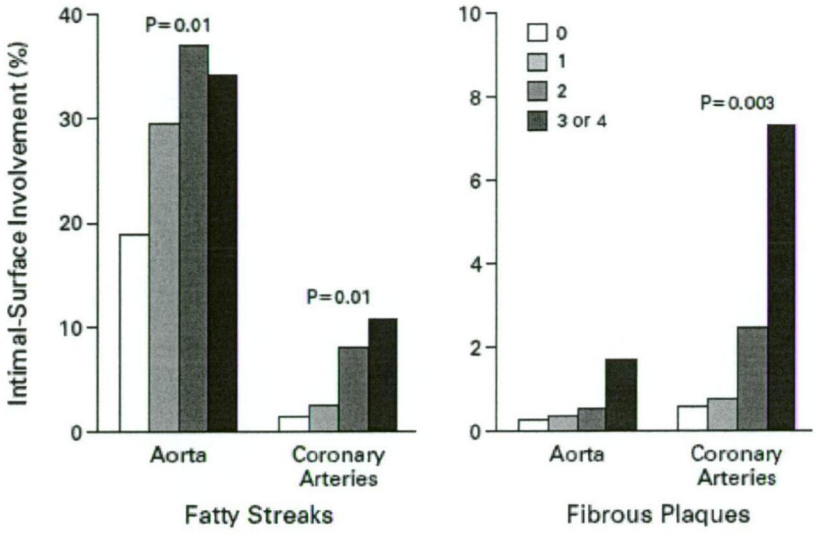


**Figure 7.** The effect of multiple CVD risk factors on the extent of atherosclerosis in the aorta and coronary arteries of autopsied youth in the Bogalusa Heart Study. From Berenson et al.[36]

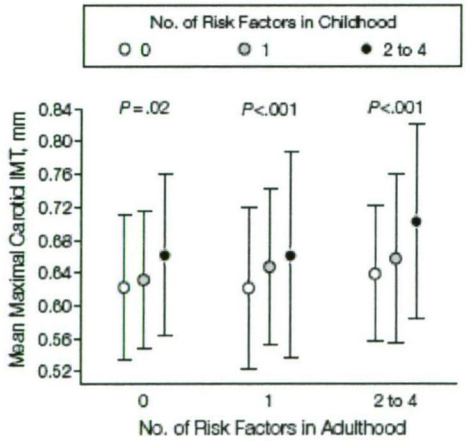


**Figure 8.** Number of current and childhood risk factors and carotid IMT in the Cardiovascular Risk in Young Finns Study. From Raitakari et al.[41]

Risk prediction estimates that combine multiple risk factors (age, sex, total cholesterol, smoking, HDL cholesterol, and systolic blood pressure) using the Framingham Risk Score have also been shown to predict carotid IMT in the Bogalusa sample.[156] Similar in concept to the Framingham Risk Score, the PDAY study group provided a risk assessment tool combining multiple risk factors (age, sex, non-HDL cholesterol, HDL cholesterol, smoking, blood pressure, obesity, hyperglycaemia) to predict advanced atherosclerosis in the coronary arteries and abdominal aorta of adolescents and young adults.[47, 157] The utility of this risk score was demonstrated in unique datasets. In the CARDIA study, risk factors measured at 18 to 30 years of age predicted coronary artery calcium in 33 to 45 year olds (for a one-point increase in risk score the odds for presence of any amount of calcium in the coronary artery increased by 10-15%), and the change in risk score between the baseline and 15-year follow-up also predicted coronary artery calcium (i.e. those who had an increase in their risk score between baseline and follow-up had higher odds of coronary calcium compared with those who remained the same or decreased their risk score).[158] In the Young Finns Study, the baseline PDAY risk score and change in the PDAY risk score between measurement time-points both independently predicted adult carotid IMT (odds of IMT >90$^{th}$ percentile or plaque were 1.12 for a one-point increase in baseline risk score and 1.04 per one-unit change in risk score between baseline and follow-up).[159] Taken together these data provide evidence that the extent of atherosclerosis increases considerably when multiple risk factors are present. These findings support the concept that multiple childhood risk factors have a synergistic effect on atherosclerosis later in life, as has been demonstrated by epidemiological studies linking multiple risk factors in middle and older age with CVD morbidity and mortality. Moreover, these data underscore the importance of prevention strategies to focus on multiple risk factors, rather than a specific risk factor.

## 1.5. IMPORTANCE OF BLOOD LIPID LEVELS IN CHILDREN AND ADOLESCENTS

As with findings in adult populations, studies involving children have provided compelling evidence that blood lipid and lipoprotein levels measured early in life play an important role in atherogenesis. For example, abnormal lipoprotein levels in children and adolescents have been associated with atherosclerosis in pathological studies;[36, 73, 113] prospective cohort studies have shown that lipoprotein levels not only track strongly from childhood and adolescence to adulthood,[160-162] but that adverse lipoprotein levels in early life may induce arterial changes that contribute to adult atherosclerosis.[34, 35, 37, 41] Given these data and findings that have demonstrated lifestyle and pharmacological intervention in children and adolescents to be effective in modifying lipoprotein levels[163-166] and improving markers of atherosclerosis,[50, 166] there is justification for identifying those at high-risk, and those who may benefit most from intervention. The following sections detail each of these areas.

### 1.5.1 BLOOD LIPID AND LIPOPROTEIN LEVELS IN CHILDHOOD, ADOLESCENCE AND YOUNG ADULTHOOD AND ARTERIAL LESIONS: PATHOLOGICAL EVIDENCE

Autopsy studies in youth and young adults have shown that high levels of total, LDL, very-low-density lipoprotein (VLDL) cholesterols, and triglycerides, and low levels of HDL cholesterol are associated with increased atherosclerosis in the aorta and coronary arteries. In their first analyses of autopsy data from 35 deceased participants, investigators from the Bogalusa Heart Study found that aortic fatty streaks were directly related to total and LDL cholesterol concentrations ($r = 0.67$ for both) and inversely associated with the ratio of HDL cholesterol to LDL plus VLDL cholesterol ($r = -0.35$), and that coronary artery fatty streaks were directly related to VLDL cholesterol concentrations ($r = 0.41$).[53] A later analysis that included autopsy specimens from 93 participants that had antemortem risk factors available confirmed the previous findings but also demonstrated a direct association between triglyceride levels and the extent of fatty streaks in the coronary arteries ($r = 0.26$) and fibrous plaques in the abdominal aorta ($r = 0.32$) and coronary arteries ($r = 0.37$), as well as a direct association of LDL cholesterol with the extent of fibrous plaques in the coronary arteries ($r = 0.32$).[36]

In the PDAY study, autopsy data from 1506 males and females showed that the extent of fatty streaks and raised lesions in the abdominal aorta and right coronary artery were directly associated with levels of non-HDL cholesterol (total cholesterol minus HDL cholesterol) and inversely associated with increasing levels of HDL cholesterol.[73] The effects were essentially similar across race and sex with the exception that a greater increase in the extent of fatty streaks in the abdominal aorta was attributable to non-HDL cholesterol levels in males compared with females. In another study by the PDAY group that examined risk factor associations with AHA lesion grade in the left anterior descending coronary artery, high non-HDL cholesterol (≥4.14 mmol/L, ≥160 mg/dL) concentrations were associated with increased odds of grade II or III lesions (OR = 2.0), grade IV or V lesions (OR = 2.6), and ≥40% stenosis (OR = 3.0). Low HDL cholesterol (<0.91 mmol/L, <35 mg/dL) concentrations were associated with increased odds of grade II or III lesions (OR = 1.6).[46] In multivariable analyses presented in a subsequent paper, high non-HDL cholesterol was significantly associated with increased odds of atherosclerotic lesions in the coronary arteries (OR = 1.41) and abdominal aorta (OR = 1.23) whereas low HDL cholesterol was no longer significant in a model that included age, sex, smoking, hypertension, obesity and hyperglycaemia.[47]

A series of publications by Napoli et al.[167-169] has demonstrated the potential of the foetal environment to affect future CVD risk. Postmortem examination of foetal aortas delivered from hypercholesterolemic (total cholesterols levels >4.65 mmol/L, >185 mg/dL) mothers contained significantly more and larger fatty streaks than aortas from normocholesterolemic mothers.[167, 168] These observations were extended in the FELIC (Fate of Early Lesions in Children) Study with data confirming that deceased children who had been exposed to maternal hypercholesterolemia during pregnancy had a more rapid rate of fatty streak formation in the aortic arch and abdominal aortas compared with children who had normocholesterolemic mothers, despite normal lipid levels at the time of autopsy.[169]

Collectively, these pathological data provide evidence that lipid and lipoprotein abnormalities in early life whether passive (in utero exposure to a hyperlipidemic environment) or direct (exposure to a hyperlipidemic environment in childhood or adolescence) are associated with accelerated atherosclerosis already by the first and second decade of life.

## 1.5.2 BLOOD LIPID AND LIPOPROTEIN LEVELS IN CHILDHOOD, ADOLESCENCE AND YOUNG ADULTHOOD AND PRECLINICAL ATHEROSCLEROSIS: EVIDENCE FROM IMAGING STUDIES

In more recent years, data has become available that link early life blood lipid and lipoprotein levels to non-invasive measures of atherosclerosis such as those outlined in Table 2. Leeson and colleagues found that total and LDL cholesterol were associated with brachial artery stiffness indices,[170] in over 300 British school children aged 9 to 11 years, suggesting that more adverse blood lipid and lipoprotein levels in the first decade of life may already be having an impact on the early stages of atherosclerosis. Another cross-sectional study by the same group, this time in children aged 13 to 15 years (some of whom had previously been studied at ages 9-11 years), found again that increasing levels of total and LDL cholesterols were significantly associated with indices of increased stiffness with the strength of effect similar to what was observed in the earlier study.[121] Total cholesterol remained significant in analyses that included all adiposity measures (BMI, percent body fat, sum of four skin folds, and waist circumference) in the model (analyses examining LDL cholesterol were not detailed).[121] In multivariable analyses of 141 Austrian 17 to 18-year old males in the ARMY study, increased HDL cholesterol levels were associated with significantly reduced odds of high IMT (OR = 0.56 for a one standard deviation increase in HDL cholesterol levels).[99] In a case-control study by Jarvisalo and colleagues, children with hypercholesterolemia (defined as total cholesterol level >6.0 mmol/L or LDL cholesterol level >4.5 mmol/L) had increased carotid (0.53 vs. 0.44 mm) and aortic (0.46 vs. 0.42 mm) IMTs compared with age-, sex-, and body-size-matched controls.[113] Another study by this group found that total cholesterol levels were inversely associated with brachial FMD (multivariable $\beta$ = -1.13 % per 1 SD increase in cholesterol levels) in a convenience sample of children aged 9 to 16 years.[171]

Not only are blood lipid and lipoprotein levels associated with concomitant markers of atherosclerosis, data from the Muscatine, Bogalusa Heart, and Young Finns studies have shown that elevated levels in childhood predict increased levels of preclinical atherosclerosis in adulthood. Using data from the Muscatine study, Davis et al. were the first to demonstrate this longitudinal relationship.[35] Total cholesterol levels measured at 8 to 18 years of age in 346 males and 379 females were significant independent predictors of high IMT (defined as the upper quarter of the IMT distribution) measured in adulthood 25 years later (male, OR = 1.53 for a one standard deviation increase; female, OR = 1.43).[35] Data on lipoproteins were

not collected at the baseline examinations but were at subsequent follow-ups. LDL cholesterol levels measured in young adulthood (measured 2 to 15 years before IMT measurement) were associated with significantly increased odds of high IMT in both sexes (males, OR = 1.39; females, OR = 1.54), while HDL cholesterol was significant only in males (OR = 0.70).[35] An earlier study of the Muscatine cohort showed a non-significant increased risk of having calcium in the coronary artery in adulthood for a one standard deviation increase in childhood total cholesterol levels for both males (OR = 2.3) and females (OR = 2.1), however it is likely these analyses were limited by low statistical power.[34]

Analyses of 486 participants from the Bogalusa Heart study who had at least three measurements of risk factors between childhood and adulthood showed that LDL cholesterol in childhood (OR = 1.42), cumulative burden of LDL (OR = 1.58) and HDL cholesterol (OR = 0.75) since childhood were independent predictors for having increased carotid IMT (top vs. lower three quartiles of carotid IMT) in young adulthood.[37] Among the risk factors examined, LDL cholesterol was shown to be the most consistent and strongest predictor of carotid IMT. A more recent analysis compared the usefulness of several childhood lipoprotein measures in predicting adult carotid IMT.[172] In separate multivariable analyses (adjusting for childhood BMI, systolic blood pressure, and length of follow-up) that examined each childhood lipoprotein measure as a predictor variable, non-HDL cholesterol (OR = 2.6), LDL cholesterol (OR = 3.0), total cholesterol/HDL cholesterol ratio (OR = 1. 8), apolipoprotein (apo) B (OR = 1.4), and apoB/apoA-I ratio (OR = 1.7) were significant predictors of adult carotid IMT. The ability to predict adult IMT from non-lipid risk factors in addition to any one of the different lipoprotein measures was not different in magnitude.

A study by Li et al.[173] found that the relationship between childhood risk factors and adult IMT varied by sex and race in the Bogalusa cohort, suggesting that targeting certain risk factors in different population groups may improve prevention programs. In multivariable analyses stratified by race and sex, significant lipid and lipoprotein predictors of carotid IMT were triglycerides and LDL cholesterol in Caucasian males; LDL cholesterol in Caucasian females; and LDL cholesterol in African-American females.[173] Although arterial stiffness,[155] common carotid lumen diameter,[174] and femoral artery IMT[175] measurements have been collected and related to adult CVD risk factors in the Bogalusa Heart Study, no studies to date have been published linking childhood risk factors to these measures; it is possible that childhood risk factors were not found to be associated with these measures. Although there are currently no longitudinal data that examine predictors of atherosclerosis

progression between childhood and adulthood, data on carotid IMT progression over six years in young adults from the Bogalusa Heart Study did not suggest that blood lipid and lipoprotein changes during this period were important.[84]

In the Cardiovascular Risk in Young Finns Study, univariable analyses of baseline (measured in 1980) and cumulative (average of levels measured at 1980, 1983, and 1986) total cholesterol, LDL cholesterol, the LDL cholesterol to HDL cholesterol ratio, and triglycerides were all shown to be directly associated with carotid IMT measured at the 2001 follow-up, but the effects were only significant in males.[41] In a multivariable model that included sex, and childhood measures of age, BMI, systolic blood pressure and smoking, baseline LDL cholesterol was a significant predictor of adult IMT ($\beta$ = 0.01 mm for a 1-SD increase in levels). The effects of childhood LDL cholesterol remained independently associated with carotid IMT when the model was further adjusted for current risk factors (beta coefficient was not shown). In a separate model examining the association of current risk variables and carotid IMT, LDL cholesterol was borderline significant ($\beta$ = 0.004 mm for a 1-SD increase in levels, P = 0.06), suggesting that childhood levels were a stronger predictor of adult IMT than those collected at the time of imaging. In two separate reports, current but not baseline LDL cholesterol and HDL cholesterol levels were found to be significant multivariate predictors of stiffness indices of the carotid artery[43] and brachial artery endothelial function respectively.[42]

Another paper from the Young Finns Study reported the effects of dyslipidemias from childhood to adulthood on adult markers of preclinical atherosclerosis.[176] Subjects with type IIb dyslipidemia (those with mean cumulative z-scores over the 90$^{th}$ percentile for LDL cholesterol and triglyceride levels in the 1980, 1983, 1986, and 2001 examinations) had increased carotid IMT compared with subjects without any dyslipidemias (type IIb = 0.621 mm vs. controls, 0.578 mm) and remained after adjustment for other risk factors (sex, age, carotid diameter, blood pressure, BMI, C-reactive protein, homocystine, insulin, glucose, family history of CVD, and smoking; adjusted IMTs were 0.607 vs. 0.579 mm). Additional analyses showed that the association between IMT with increasing number of non-lipid risk factors or presence of the metabolic syndrome was stronger in those with type IIb dyslipidemia than in controls, suggesting that the increased IMT in those with type IIb dyslipidemia was partly explained by their increased vulnerability to the effects of non-lipid risk factors and the metabolic syndrome.[176] The association between apoB and apoA-I, the major constituent proteins of LDL cholesterol and HDL cholesterol respectively, in

childhood with preclinical markers of atherosclerosis were recently examined in the Young Finns cohort.[177] Childhood levels of apoB and apoB/apoA-I ratio were directly associated and apoA-I inversely associated with adult carotid IMT. In analyses using brachial artery endothelial function as the outcome measure, apoB and apoB/apoA-I ratio were inversely associated and apoA-I directly associated with adult FMD. In a multivariable model that included non-lipid risk factors and adulthood apolipoproteins, both apoB and apoA-I were significant independent predictors of adult carotid IMT (apoB, β 0.017 mm for a 1-SD increase; apoA-I, -0.011 mm) and brachial FMD (apoB, β -0.38 % for a 1-SD increase; apoA-I, 0.47 %). Interestingly, a multivariable model that contained the childhood apoB/apoA-I ratio in addition to blood pressure and smoking significantly increased the prediction of high carotid IMT in adulthood compared with a model that included LDL/HDL cholesterol ratio in addition to blood pressure and smoking. These data suggest that measurement of apoB and apoA-I in childhood may be more useful in paediatric CVD risk assessment than serum concentrations of LDL and HDL cholesterols. Publications linking childhood risk factors to 6-year IMT progression in young adulthood in this cohort are currently pending.

In the CARDIA study, multivariable models showed that for each 30mg/dL increase in LDL cholesterol levels measured at 18 to 30 years the odds of calcium being present in the coronary arteries after 10-years (OR = 1.71)[74] and 15-years of follow-up (OR = 1.41)[71] significantly increased. Of interest, was that baseline LDL cholesterol levels were stronger predictors of 10-year and 15-year coronary calcification than contemporary risk factors measured at the time of scanning. In the 15-year follow-up, baseline LDL cholesterol levels predicted coronary calcium regardless of 15-year change in levels, and 15-year change in LDL cholesterol levels were not related to calcification independently of baseline levels (baseline, OR = 1.42; 15-year change, OR = 1.09). These data from the CARDIA study demonstrated that LDL cholesterol levels measured in young adulthood predicted the presence of calcified plaque better than concurrent levels, and regardless of the change in levels that had occurred in the 15-year interval.

Taken together the above evidence highlights that while childhood and young adulthood levels of blood lipid and lipoprotein levels may be relatively low, they are, and in some cases more, important in the prediction of markers of atherosclerosis in young to middle adulthood suggesting that earlier risk assessment and preventive efforts targeted at the young may prove beneficial in reducing CVD burden.

### 1.5.3 FAMILIAL AGGREGATION OF ELEVATED LIPID AND LIPOPROTEIN LEVELS AND THEIR RELATIONSHIP TO ATHEROSCLEROSIS

Children of families in which adult members have hypercholesterolemia tend to have more adverse lipid and lipoprotein profiles compared to their peers.[178] The *aggregation* of more adverse lipid profiles in these children is a result of both shared genetic (inherited) factors and shared environmental (lifestyle) factors. Inherited forms of lipid disorders that present in youth include familial hypercholesterolemia (FH), and familial combined hyperlipidemia (FCH);[179] both occur as a result of genetic defects in lipid metabolism pathways. For example, FH results from a single gene defect that affects LDL cholesterol receptors on the surface of hepatocytes impairing the clearance of circulating LDL cholesterol[180] that leads to severely elevated LDL cholesterol levels from birth onwards.[181] Familial combined hyperlipidemia results from hepatic overproduction of VLDL cholesterol due to what is thought to be the influence of multiple genetic factors.[182] The phenotype expression of FCH may include elevated LDL cholesterol alone (type IIa) or in combination with hypertriglyceridemia (type IIb), or acceptable LDL cholesterol with hypertriglyceridemia (type IV).[180] Both FH and FCH have been attributed to premature CVD,[183-185] in some cases before the age of 20.[186] Multiple studies have also compared non-invasive measures of atherosclerosis in youth with and without these lipid disorders. A study by Gidding et al.[187] found that youth with FH had detectable coronary calcium in the second decade of life, long before it would typically present. Children and adolescents with the heterozygous form of FH show impaired endothelial-dependent FMD[188, 189] and increased carotid IMT[190] compared with matched controls. In case-control studies, youth with FCH have also been shown to have impaired brachial FMD,[191, 192] and to have abnormalities in coronary flow regulation.[193] Data on carotid IMT in youth with FCH are not currently available, however, a recent publication from the Young Finns study showed that youth with type IIb FCH had increased carotid IMT (0.62 vs. 0.58 mm) but not impaired brachial FMD or carotid compliance in adulthood compared with those who did not have any form of FCH.[176] It should be noted that while children and adolescents with FH and FCH are at high risk for CVD, not all of these youth have accelerated atherosclerosis or reduced life expectancy.[194, 195] Moreover, while FH and FCH are the most common inherited lipid disorders, they do not account for all children and

adolescents with elevated blood lipid and lipoprotein levels nor do they explain the majority of premature CVD.[196]

Studies that collect phenotypic information from families with different degrees of genetic similarity (low similarity in the case of spouses or adoptees to high similarity in the case of monozygotic twin-pairs) have demonstrated that both shared genetic backgrounds and shared environments help explain the variance in blood lipid and lipoprotein values. These studies show heritability (i.e. the percentage of total variance attributed to shared genetics) to be in the order of 40 to 60%,[197] with shared environment tending to explain less variance in total and LDL cholesterol compared to HDL cholesterol and triglyceride,[198] suggesting a greater role for family environment and lifestyle factors in the modification of HDL cholesterol and triglyceride levels. These findings are not surprising given the concomitant increase in the prevalence of youth with low HDL cholesterol and high triglycerides in recent history due to the obesity epidemic. It is evident therefore, that children of families in which premature CVD or hypercholesterolemia occurs are at an elevated risk of adverse blood lipid and lipoprotein levels – promoted through the aggregation of both shared genes and shared environment.

The evidence outlined in the previous sections suggests that child and adolescent lipid and lipoprotein levels are strong, and in most cases, independent predictors of current and future atherosclerosis burden and that raised blood lipid and lipoprotein levels tend to aggregate within families. The following section details evidence of the effect of lipid and lipoprotein modification in children and adolescents on markers of atherosclerosis.

### 1.5.4 DOES THE MODIFICATION OF BLOOD LIPID AND LIPOPROTEIN LEVELS IN CHILDREN AND ADOLESCENTS HAVE AN EFFECT ON ATHEROSCLEROSIS?

A recent systematic review by the US Preventive Service Task Force provided an elegant summary of the effectiveness of drug, diet, exercise, and combination therapies for treating dyslipidemia in children and adolescents.[199] The available evidence summarised by this group[199] and others[200, 201] suggests a benefit of statin drugs among children with FH in reducing total cholesterol, LDL cholesterol, and apoB levels whereas HDL cholesterol and apoA-I levels increase; diet treatments in those children and adolescents with or without FH appear to reduce total and LDL cholesterol; and a benefit of exercise treatment for increasing

HDL cholesterol in children and adolescents without FH. While these studies provide evidence of improved lipid levels, they do not demonstrate effectiveness of such treatments in reducing the optimal outcome of CVD morbidity and mortality. In the absence of such data, preclinical markers of vascular function and structure (as outlined in Table 2) that are directly related to the atherosclerotic process are increasingly being used as intermediary outcomes.

In children with FH, pharmacological intervention with statin drugs (simvastatin and pravastatin) reduced total and LDL cholesterol levels, improved endothelial function,[188, 202] and regressed carotid IMT[166] compared with matched placebo groups. The study by de Jongh et al.[188] showed that changes in total and LDL cholesterol after 28 weeks of therapy were responsible for part of the observed increase in endothelial function (correlation between change in total cholesterol and LDL cholesterol with change in FMD were $r = -0.31$, and $r = -0.31$ respectively). Interestingly, Rodenburg et al.[203] demonstrated that earlier initiation of statin therapy in childhood delayed the progression of carotid IMT into adolescence and young adulthood.

The STRIP study, a clinical trial of dietary counselling in infancy aimed at reducing saturated fat intake,[204] has shown the intervention group to have more favourable total and LDL cholesterol values during the first 3 years, and again at age 5, 7, and 14 years,[51, 164, 205, 206] but does not harmfully influence children's growth.[207, 208] The effect on endothelial function was also demonstrated with boys in the intervention group at the age of 11 years shown to have significantly higher endothelium-dependent FMD compared with control boys (9.6 vs. 8.4%).[50] This difference remained significant after adjustment for current total or LDL cholesterol levels but became non-significant after adjusting for mean total cholesterol levels measured during the first 3 years, suggesting that the difference between the intervention and control groups was explained in part by the diet-induced reduction in cholesterol levels.[50] These studies provide evidence that the modification of lipid and lipoprotein levels in children and adolescents does appear to impact on surrogate markers of atherosclerosis. While preliminary, these data suggest that there may be a benefit of beginning preventive and intervention efforts in childhood and adolescence as a means of reducing CVD later in life.

## 1.5.5 LEVELS AND PATTERNS OF LIPIDS AND LIPOPROTEINS IN CHILDREN AND ADOLESCENTS

Research has identified two periods in childhood and adolescence when lipoprotein profiles undergo a substantial shift: during the first two years of life, and during sexual maturation. Observations on infants, pre-school children and school-aged children from the Bogalusa Heart Study[209] and Lipid Research Clinics (LRC) Prevalence Study[210] indicate that the most dramatic shift in lipid and lipoprotein levels occurs during the first two years of life when concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides increase to approach young adult levels. This shift parallels the sudden growth spurt that occurs during this period. After this time, blood lipid and lipoprotein levels remain relatively stable until the onset of puberty. Rapid alterations in growth and sexual maturation during puberty bring dynamic sex- and race-related changes in lipid and lipoprotein levels. Data from the Bogalusa Heart study showed that total cholesterol levels decline at the onset of puberty in Caucasian and African-American males and females. This decline in Caucasian males reflects a drop in HDL cholesterol levels, whereas a drop in LDL cholesterol levels in Caucasian females and African-American males and females is responsible for the decline in total cholesterol levels. At maturation, LDL cholesterol levels begin to rise but HDL cholesterol continues to fall in Caucasian males. Triglyceride levels tend to rise during puberty and level-off at maturation in females, whereas levels continue to rise in males. A review of blood lipid and lipoprotein distributions in childhood and adulthood using data on 60,494 observations from 26 countries showed similar trends to those reported in the Bogalusa Heart Study with changes occurring from 10 to 13 years of age.

A number of population-based studies have provided data on the distribution of lipid and lipoprotein levels in youth. An elegant study by Knuiman et al.[211] averted difficulties of comparing distributions and levels across different populations by using standardised methodology to assess total cholesterol and HDL cholesterol in boys aged 8 to 16 years from 16 countries. The results showed that every population (even within countries) has its own unique cholesterol distribution, and that this distribution tends to reflect the adult CVD mortality in the region, with the highest total cholesterol concentrations observed in populations from the US and Northern Europe and the lowest observed in populations from Western Africa. A study by Dwyer et al.[212] that compared HDL cholesterol levels in children from Australia, the US, and Japan found that Japanese children had significantly higher HDL

cholesterol levels compared with their Australian and US contemporaries. Moreover, the decline in HDL cholesterol observed in males at the onset of puberty was much more pronounced in Australian (-15%) and US boys (-9%) than Japanese boys (-4%). They concluded that the differences observed may help to explain why CVD mortality in Japan is low compared with that in other developed countries. Differences observed across geographical regions are likely both genetic and environment-based. Evidence for genetic-based differences are provided by US data from the NHANES,[213] the Bogalusa Heart Study,[209] and the Cardiovascular Health in Children Study[214] that have demonstrated differences in blood lipid and lipoprotein levels by ethnicity with African-American children tending to have higher total and HDL cholesterol levels compared with Caucasian. While data from the aforementioned Knuiman[211, 215] and Dwyer[212] studies provided evidence for possible environmental differences between populations in their studies. For example, Knuiman found that the percent of energy intake from saturated fat was substantially higher in boys from countries where total cholesterol levels were higher.[215] In the Dwyer paper, Japanese children participated in more physical activity per week compared with Australian children.[212] Collectively, these data suggest that lipid and lipoprotein distributions differ considerably by geographical region and by age, sex, race, and pubertal-status.

Routine observations of lipid and lipoprotein levels in children and adolescents at different periods using standardised methodology allows secular trends to be observed. Reporting risk factor trends between 1966-1970 and 1988-1994, Hickman et al.[213] reported a secular decline of 0.18 mmol/L (7 mg/dL) in mean total cholesterol levels in 12 to 17 year old adolescents using NHANES data. The separate contribution of LDL and HDL cholesterol could not be deduced from these data, since lipoprotein measures were not collected from the earlier survey. A more recent update by Ford et al.[216] examined temporal trends between 1988-1994 and 1999-2000 in the NHANES data. A significant decline in triglyceride levels (-0.24 mmol/L; -8.8 mg/dL) was observed over this time but total cholesterol, LDL cholesterol, and HDL cholesterol levels remained largely unchanged. Data from the Young Finns Study found evidence for declines in total cholesterol (-0.41 mmol/L; -16 mg/dL), LDL cholesterol (-0.21 mmol/L; -9 mg/dL) and HDL cholesterol (-0.28 mmol/L; -11 mg/dL) levels, and an increase in triglyceride levels (0.13 mmol/L; 12 mg/dL) in 15 and 18 year olds between 1980 and 1992.[217] Significant declines in total cholesterol and HDL cholesterol and an increase in triglyceride levels were also confirmed in 24 year olds from this cohort between the 1986 and 2001 surveys.[218] Data on children from the Bogalusa Heart Study were consistent with those

from adolescents in the Young Finns Study. For example, Gidding et al.[219] showed that HDL cholesterol levels had decreased (-0.16 to 0.34 mmol/L; -6 to 13 mg/dl) and triglyceride levels had increased (0.09 to 0.41 mmol/L; 8 to 36 mg/dl) in 7 to 9 year olds between 1973 and 1992. While the reasons for these changes are not completely understood,[220] the observed reductions in total cholesterol and LDL cholesterol may be explained by reductions in cigarette smoking and saturated fat intake.[221-225] Whereas the reduction in HDL cholesterol levels and increase in triglyceride levels may be attributed to the well-documented increase in prevalence of child and adolescent overweight and obesity, particularly since the 1980s.[226-230] The available data from a number of cohort studies have shown that the most consistent predictor of changes in lipid and lipoprotein levels is changes in adiposity; with increased adiposity associated with a subsequent decrease in HDL cholesterol levels and an increase in triglyceride levels.[161, 219, 223, 231] Obesity-associated dyslipidemia is a major concern facing contemporary youth. Not only is it associated with a more atherogenic lipid profile, the constellation of obesity-associated dyslipidemia with other indicators of the metabolic syndrome have been linked with markedly increased risk of type II diabetes and CVD mortality.

### 1.5.6 TRACKING OF BLOOD LIPID AND LIPOPROTEIN LEVELS FROM CHILDHOOD OR ADOLESCENCE INTO ADULTHOOD

Are blood lipid and lipoprotein levels measured in childhood or adolescence good indicators of levels in adulthood? This question has been examined in a number of prospective cohort studies conducted over the past three decades.[160-162, 223, 224, 231-245] The term *tracking* has been used to describe analyses of this type. Essentially, tracking denotes the degree of consistency over time of an attribute, and is used to determine the ability to predict future values from measurements taken early in life.[246] For diseases that have a long latency before clinical manifestation and have established risk factors (such as atherosclerosis), studies of modifiable risk factor tracking provide an insight as to whether identifying high-risk individuals at one time point may be useful in identifying those at highest risk of developing the disease at a later time point. There are important public health implications if risk factors are found to track. For example, prevention or treatment programs could begin earlier and targeted to the populations or individuals at highest risk of the disease.

The studies that have examined tracking of blood lipid and lipoprotein levels between childhood or adolescence and adulthood[160-162, 223, 224, 231-245] are extensively covered in Chapter 3 of this thesis but a summary is provided here. These studies suggest moderate to strong tracking of total cholesterol (correlation coefficients, r, ranging from 0.33-0.73), LDL cholesterol (range r = 0.40-0.72), and HDL cholesterol levels (range r = 0.20-0.71), and low to moderate tracking of triglyceride levels (range r = 0.11-0.57) between childhood or adolescence and adulthood. While these studies suggest that children with high lipid and lipoprotein levels have a greater risk of having elevated adult levels than their peers with lower levels, they show that a substantial proportion of these children do not have adult levels that meet cut-points for intervention.

Key limitations of the tracking literature includes use of only one measurement to assign risk status at both baseline and follow-up in most studies, non-standardised definitions of the at-risk group, and the paucity of data examining both tracking of fasting LDL cholesterol, HDL cholesterol and triglycerides levels from large population-based samples, and lifestyle factors that may have contributed to individuals changing their risk status between childhood or adolescence and adulthood.

## 1.6. PRIOR AND CURRENT GUIDELINES FOR SCREENING AND MANAGEMENT OF LIPID DISORDERS IN CHILDREN AND ADOLESCENTS

The US National Cholesterol Education Program (NCEP) expert panel published consensus guidelines in 1992 for the detection and treatment of dyslipidemia in children and adolescents aged 2 to 19 years. This was in response to accumulating laboratory, clinical, pathological, and epidemiological evidence available at the time, that demonstrated elevated levels of total and LDL cholesterol in adults to be associated with cardiovascular outcomes, pathological data suggesting the presence of dyslipidemia in youth to be associated with early atherosclerotic lesion development, the tracking of lipid and lipoprotein levels from childhood to adulthood, and family history of premature CVD to be useful in identifying youth with elevated LDL cholesterol levels.[196] The panel recommended that a combined strategy consisting of both a population and an individualised approach be adopted in the aim of lowering total and LDL cholesterols. The purpose of the population approach was to lower the average blood lipid levels of all youth through providing population-wide dietary recommendations; while the purpose of the individualised approach was to identify children at highest risk of CVD by selective screening blood lipid and lipoprotein levels in those with family history of premature CVD and/or parental hypercholesterolemia. The selection, screening, and therapeutic recommendations were determined by a series of algorithms (Figure 9, and Figure 10).

For children and adolescents with a parental or grandparental history of premature (<55 years) CVD (coronary atherosclerosis, coronary procedure [balloon angioplasty or bypass surgery], myocardial infarction, angina pectoris, peripheral vascular disease, cerebrovascular disease, or sudden death), the panel recommended a fasting lipoprotein analysis. For children and adolescents with parental hypercholesterolemia (total cholesterol level >6.20 mmol/L; 240 mg/dL), the panel recommended screening for total cholesterol levels in the first instance (Figure 9). Three levels for classification of total cholesterol levels were provided. Total cholesterol levels <4.40 mmol/L (<170 mg/dL) were classified as acceptable, levels from 4.40 mmol/L to 5.17 mmol/L (170-200 mg/dL) were considered borderline-high, and values that exceeded 5.20 mmol/L (200 mg/dL) were considered as high. In those with borderline-high levels, a repeat measurement was emphasised. If the average of both measurements was <4.40 mmol/L (<170 mg/dL) or the initial measurement

was acceptable, repeat measurements were recommended within five years. For those that remained in the borderline-high category after repeat analysis, no recommendation was provided. If the average of the two measurements exceeded 5.20 mmol/L (200 mg/dL), or for those classified as having high total cholesterol levels at the initial measurement, analysis of fasting lipoprotein levels were to be obtained.

For those where fasting lipoprotein analysis was emphasised, further evaluation and treatment recommendations were then based on LDL cholesterol levels (Figure 10). Classification was based on the average of two measurements to account for within-person variability. Those with LDL cholesterol levels <2.85 mmol/L (<110 mg/dL) were defined as acceptable and required lipoprotein analysis again within five years. Those with values from 2.85 to 3.35 mmol/L (110-130 mg/dL) were defined as borderline-high, advised to follow the dietary guidelines set out for the population-based approach and to have their lipoprotein status re-evaluated in one year. Those with levels above 3.35 mmol/L (1.30 mg/dL) were considered as high, and advised to progress from the 'step-one' diet, which was consistent with the dietary guidelines for the general population, followed by the 'step-two' diet which placed further restrictions on saturated fat and cholesterol intake. The panel recommended the consideration of pharmacological therapy in youth aged 10 years and older if, after an adequate trial (6 months to 1 year) of diet therapy (step-one progressing to step-two), LDL cholesterol levels were above 4.90 mmol/L (190 mg/dL) or were above 4.10 mmol/L (160mg/dL) together with either a positive family history of premature CVD *or* two or more other current CVD risk factors (cigarette smoking, elevated blood pressure, low HDL cholesterol levels, severe obesity, diabetes mellitus, or physical inactivity).

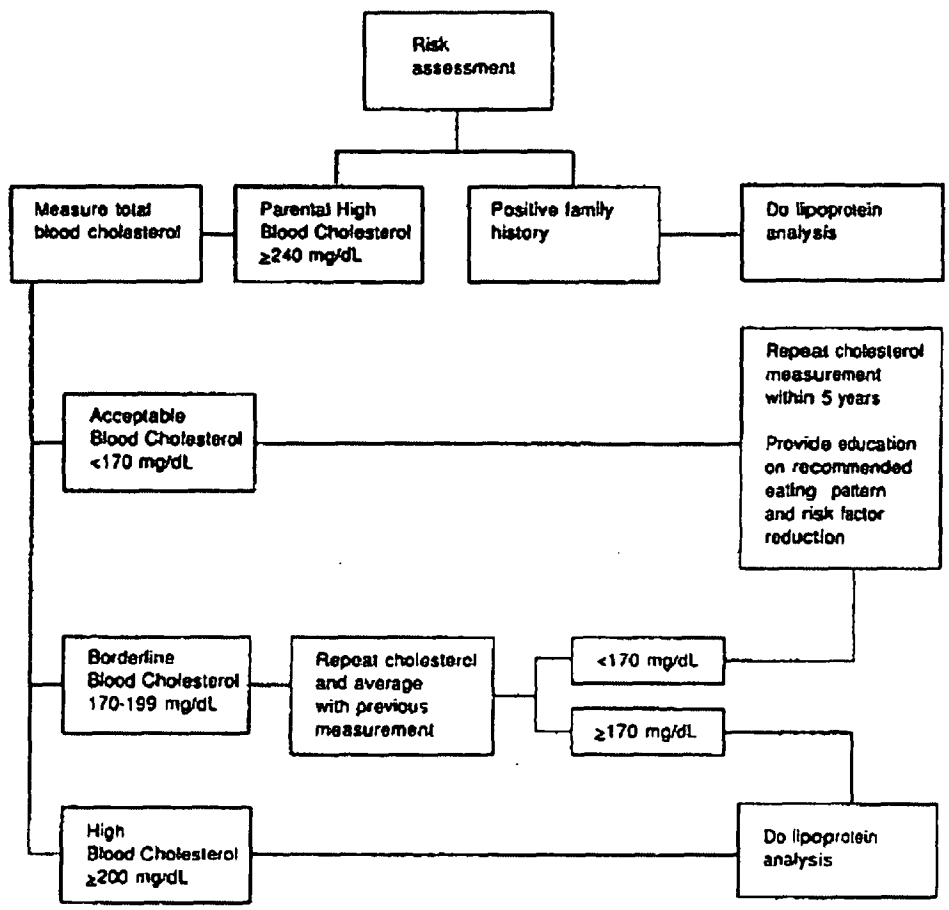


**Figure 9.** Individualised approach of risk assessment of paediatric blood cholesterol levels according to the NCEP guidelines.[196]

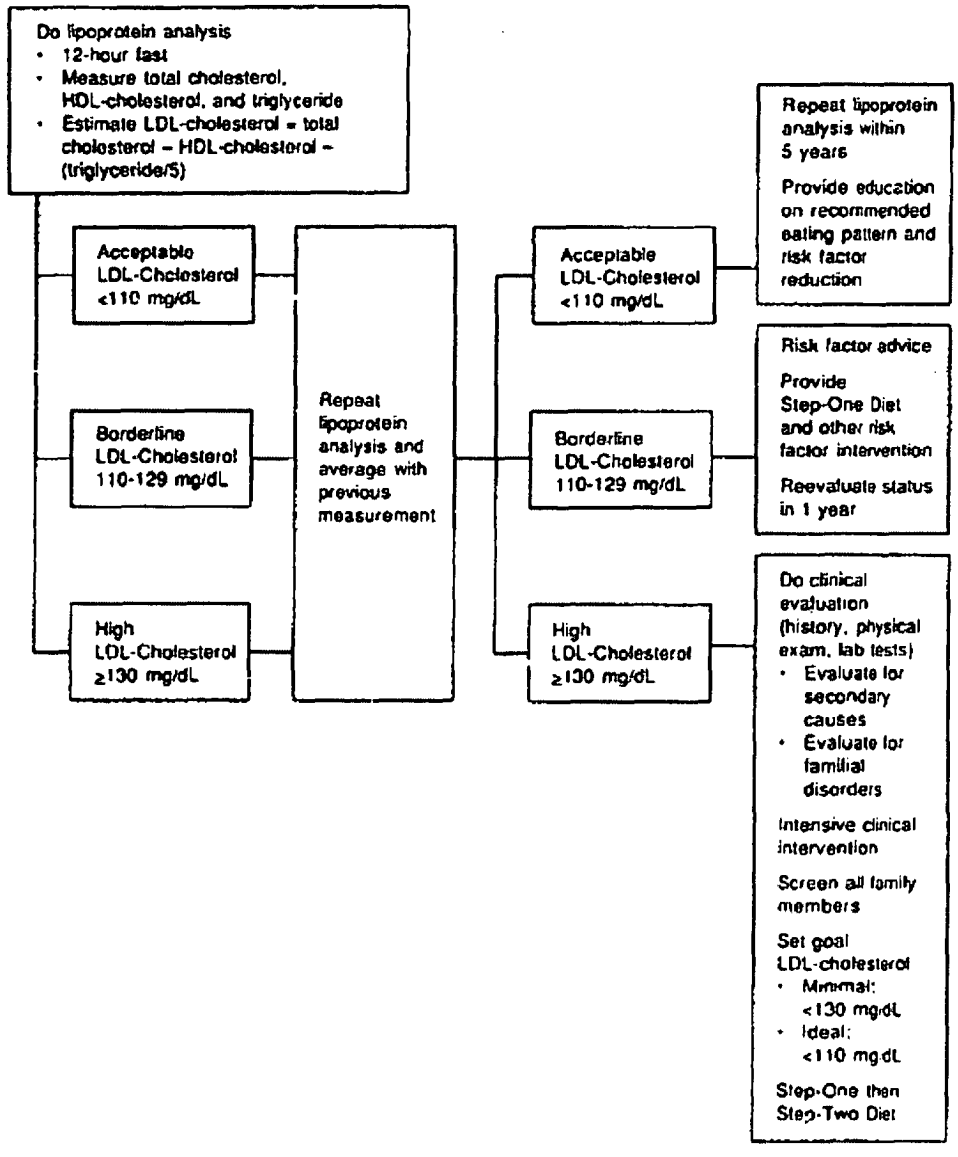


**Figure 10.** Classification and treatment recommendations based on paediatric LDL cholesterol levels from the NCEP using the individualised approach.[196]

Universal (or population-wide) screening of blood lipid levels in all children and adolescents was not recommended due to reservations from the panel with regard to (1) predictiveness (not all youth with high-risk levels become adults with high-risk levels); (2) psycho-social aspects of *labelling* youth with lipid disorders as having a 'disease'; (3) there being sufficient time to begin lipid-lowering therapies in adulthood, and (4) insufficient evidence concerning the long-term safety and efficacy of pharmacological therapy in youth.

In 1998, the American Academy of Pediatrics (AAP) Committee on Nutrition issued a statement on cholesterol in childhood that adopted all of the attributes of the original NCEP guidelines, but made some minor modifications to nutrient recommendations.[247] In 2003, the AHA issued guidelines for the primary prevention of CVD beginning in childhood.[248] Again, these essentially followed the recommendations set forth by the 1992 NCEP consensus. The AHA guidelines further added that pharmacologic intervention for dyslipidemia be accomplished in collaboration with a physician experienced in the treatment of cholesterol disorders in youth. This report also recommended therapeutic lifestyle changes (weight management with appropriate diet and increased physical activity, decreased intake of simple sugars) in those with elevated triglyceride levels (>1.50 mmol/L; 150mg/dL) and/or low HDL cholesterol levels (<0.90 mmol/L; 35 mg/dL). No pharmacologic intervention was recommended for those children with elevated triglyceride or low HDL cholesterol levels. In addition, the report suggested that the average of three fasting lipid profiles be used before risk was classified, and emphasised weight management and increased physical activity in those with high total or LDL cholesterol levels.

In the time since the commencement of this dissertation, new guidelines for testing and treating lipid disorders have been published. In 2007, the AHA updated recommendations for pharmacologic therapy in children and adolescents with high-risk lipid abnormalities,[249] and in mid 2008, the AAP issued a revised set of guidelines on cholesterol in childhood that superseded those issued in 1998.[220] The updated guidelines continued to emphasise both the population-based approach and the individualised approach, but provided recommendations for more comprehensive screening (extending lipid and lipoprotein analysis to youth with other CVD risk factors – particularly overweight and obesity, but also hypertension, cigarette smoking or diabetes), age- and sex-specific decision cut-points, the inclusion of routine HDL cholesterol and triglyceride analysis in addition to LDL cholesterol, improved quality of fat in the diet rather than reducing total fat consumption, a lowering of the age at which pharmacologic intervention may be considered (from 10 to 8 years), and the

inclusion of statins as potential first-line pharmacologic agents, with the target LDL cholesterol level reduced from <4.1 mmol/L (160 mg/dL) to 'minimum' <3.3 mmol/L (<130 mg/dL) and 'ideal' <2.85 mmol/L (110 mg/dL) levels. A comparison of the recommendations on blood lipid screening and management in paediatrics issued by NCEP in 1992 and revised by the AAP in 1998 with recommendations set-forth in the 2008 revision from the AAP are presented in Table 3. The revised guidelines from both groups were issued in concert with a systematic review from the US Preventive Services Task Force[250] and in reply to recent calls from this group, the American Heart Association,[249] and others,[220, 251-253] that outlined challenges with the existing guidelines and had called for revision based on data that had become available since the guidelines were first published.[196] It should also be noted that the National Heart Lung and Blood Institute (NHLBI) will soon release recommendations from the Pediatric Cardiovascular Risk Reduction Initiative that aims to address overall CVD risk factor identification and risk reduction in children (http://www.nhlbi.nih.gov/guidelines/cvd_ped/index.htm).

**Table 3.** Comparison of paediatric guidelines on lipid screening and treatment from 1992 to 2008

| Recommendation | NCEP, 1992[196] and AAP, 1998[247]* | AAP, 2008[220] |
|---|---|---|
| Screening | **Population approach**<br>No screening recommended | **Population approach**<br>No screening recommended |
| | **Individual approach**<br>Measure non-fasting TCH or fasting lipids if:<br>1. family history (parent or grandparent) of premature CVD ≤55 years, or<br>2. at least one parent with known high cholesterol levels ≥6.20 mmol/L (≥240 mg/dL), or<br>3. optional with unknown/unobtainable family history, or<br>4. optional given personal risk factors (cigarette smoking, high blood pressure, consumption of excessive saturated fat, overweight, those with medical conditions such as diabetes). | **Individual approach**<br>Measure fasting lipid profile if:<br>1. family history (parent and/or grandparent not-specified) of premature CVD ≤55 years for males and ≤65 years for females, or<br>2. family history (parent and/or grandparent not-specified) of dyslipidemia (values not specified), or<br>3. recommended with unknown family history, or<br>4. Recommended given personal risk factors (overweight or obese, BMI ≥85$^{th}$ percentile; hypertension, blood pressure ≥95$^{th}$ percentile; cigarette smoking, diabetes mellitus). |
| | Start age: ≥2 years.<br>Screening interval: at least every 5 years<br>No specific recommendations for high TG levels or low HDL-C levels. | Start age: ≥2 years, no later than 10 years<br>Screening interval: every 3-5 years<br>HDL-C and TG considered in recommendations for those who are overweight or obese. |
| Cut-points | Single set of cut-points (for all ages and both sexes) for total cholesterol and LDL cholesterol. | Age- and sex-specific values corresponding to norms[210] (>95$^{th}$ percentile for TCH, LDL-C and TG, <5$^{th}$ percentile for HDL-C). |
| Diet | **Population approach**<br>NCEP Step-One Diet (total fat <30% of total calories; saturated fat <10% of total calories; dietary cholesterol <300 mg/day). The AAP later added that total fat should be no less than 20% of total calories.[247] | **Population approach**<br>Follow Dietary Guidelines for Americans (saturated fat <7%, trans fat <1% of total calories; dietary cholesterol <200 mg/dL; fibre intake equal to child's age plus 5 g/day up to 20 g/day at age 15). |
| | **Individual approach**<br>Nutritional therapy: begin at 2 years of age<br>1. NCEP Step-One Diet (outlined above)<br>2. If Step-One Diet not effective after 3-mo, progress to Step-Two Diet (saturated fat <7% of total calories; dietary cholesterol <200 mg/day) | **Individual approach**<br>Nutritional therapy:<br>1. Reduced-fat milk to begin at 1 year for children at risk of overweight or obesity or who have family history of obesity, dyslipidemia, or CVD.<br>2. Follow Dietary Guidelines for Americans when ≥2 years of age.[254, 255] |
| Physical activity | No specific guidelines, but regular exercise encouraged. | No specific guidelines, but increased physical activity encouraged for treatment of high TG levels and low HDL-C levels in overweight or obese. |
| Pharmacology | **Individual approach**<br>Consider drug therapy if children ≥10 years of age, after an adequate trial of diet therapy (6 mo to 1 year) and either:<br>1. LDL-C remains ≥4.90 mmol/L (≥190 mg/dL), or<br>2. LDL-C remains >4.10 mmol/L (>160 mg/dL) and<br>a. family history of premature CVD, or<br>b. ≥2 CVD risk factors | **Individual approach**<br>Consider pharmacologic intervention if children <8 years of age and LDL-C >12.90 mmol/L (>500 mg/dL), or ≥8 years of age and either:<br>1. LDL-C ≥4.90 mmol/L (≥190 mg/dL), or<br>2. LDL-C >4.10 mmol/L (>160 mg/dL) and<br>a. family history of premature CVD, or<br>b. ≥2 CVD risk factors, or<br>c. ≥3.35 mmol/L (≥130 mg/dL) if diabetes mellitus is present |
| | Use bile acid-binding resins as first-line treatment, Niacin (nicotinic acid) under supervision of lipid specialist. | Statins, bile acid-binding resins, cholesterol-absorption inhibitors as potential first-line agents, fibrates under supervision of paediatric lipid specialist. |

NCEP = National Cholesterol Education Program; AAP = American Academy of Pediatrics; CVD = cardiovascular disease; TCH = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; BMI = body mass index

*Guidelines issued by the AAP were consistent with the NCEP guidelines with exceptions noted in the table.

### 1.6.1 CHALLENGES WITH THESE GUIDELINES

Reports from the AHA[249] and the US Preventive Services Task Force[199] have provided recent and thorough reviews examining the application of the NCEP and AAP guidelines in view of newer evidence that has become available since they were first issued. The areas outlined for revision can be loosely categorised as follows: screening, guideline compliance, and management (efficacy, effectiveness, and type of therapeutic intervention). In the following section, issues concerning screening will be summarised as they are key to the aims of this thesis.

### 1.6.2 SCREENING

*How many children and adolescents would be screened?*

Using data from the LRC Prevalence Study, the NCEP panel estimated that 25.1% of youth aged 2 to 19 years would be screened according to the individualised approach using a combination of positive family history of premature CVD (5.6%) and parental hypercholesterolemia (19.5%). However, a number of population-based studies with medium to large sample sizes have suggested that the number of youth requiring lipid screening to be in the order of 25 to 55%.[256-265] In the largest study of these, Dennison et al., found that 38% of 10,457 youth aged 2 to 19 years would meet screening criteria, 27% due to family history of premature CVD and 11% due to parental hypercholesterolemia. Griffin et al. reported 35% of pre-pubertal, predominantly Caucasian children aged 2 to 13 years would meet the screening criteria. A report from O'Loughlin et al. on data from the Quebec Child and Adolescent Health and Social Survey suggested that targeted screening would be evident for 26% of 3665 predominantly French-Canadian youth aged 9, 13, and 16 years. Because only parental history of premature CVD was collected, this was likely an underestimate of the actual amount that would have met the NCEP/AAP criteria. A study of 1140 fifth-grade students in a school-affiliated, health-education program in Arizona found that 54% had a family history of hypercholesterolemia or MI. While the wide discrepancy in the proportions of youth that would be subject to lipid screening is likely a function of discordant definitions of family history of premature CVD, the threshold used to denote hypercholesterolemia, population differences, or a combination of these, it is evident from these data that a higher proportion of children and adolescents would be screened than was first estimated.[199, 220]

*What is the diagnostic accuracy of the selective screening approach?*

A perfect, cost effective, screening tool would be able to identify all of those children with high-risk blood lipid and lipoprotein levels, while at the same time not unduly subject those children that do not have high blood lipid or lipoprotein levels to blood draws. Diagnostic tests are often examined in terms of *sensitivity*, the proportion of diseased persons that are correctly identified as positive by the screening test; and *specificity*, the proportion of healthy (non-diseased) persons that are correctly identified as negative by the screening test. Ideally, a screening test should have both high sensitivity and high specificity, or a reasonable trade-off between the two values that is considered 'acceptable'. In the case of testing for blood cholesterol levels in youth, the 'disease' is high blood lipid or lipoprotein levels, which is only a surrogate marker for the risk of the true disease outcome, atherosclerosis and CVD.[220]

A number of studies have examined the diagnostic utility of the individualised screening approach based on family history[256, 257, 259, 261, 262, 264-275] recommended in the previous,[196, 247] and adopted with other triggers for screening in the updated AAP guidelines.[220] The recent systematic review from the US Preventive Services Task Force of these studies reported that a substantial proportion of youth with elevated lipid and lipoprotein level were missed using the selective screening approach based on family history.[199] Sensitivity estimates in these studies ranged from 10 to 83% with most studies suggesting that 30 to 60% of youth with high-risk levels would not be identified. Specificities also varied substantially from 24 to 79%; the lowest reported in a sample of predominantly Caucasian fifth-grade students,[259] and the highest reported in a sample of mostly Hispanic senior high-school students.[274] It should be noted that these studies used varied definitions of what constituted positive family history (first- or second-degree relatives, age definition of 'premature', total cholesterol level used to denote the presence of family history for hypercholesterolemia). Findings from Friedman et al.[276] reporting on data from the Princeton LRC Prevalence Follow-up Study deserve special mention. This study measured 900 participants aged 5 to 19 years at baseline and again when they were aged 28 to 48 years to examine the diagnostic utility of the selective screening approach in identifying children who would have high total cholesterol or LDL cholesterol levels in adulthood. They found sensitivities of 46% for both total and LDL cholesterol and specificities of 84% and 82% respectively for the selective screening approach endorsed by NCEP and the AAP. Interestingly, they showed that a universal (population-wide) approach to screening to provide comparable prediction of adult values when compared with the more-focused

screening of youth with positive family history, illustrating limited benefits of targeted screening for this purpose.

Limitations in accurately obtaining full family history of premature CVD and hypercholesterolemia have also been highlighted,[199, 220, 249] which may also have affected diagnostic utility of this screening approach. For example, family history may not be known by the parents, both parents may not be available, the parents may not have had their cholesterol levels checked themselves, or those parents that have had their cholesterol level measured may not know their results.[260, 277] Another concern is how well total cholesterol levels sampled from those youth with family history of hypercholesterolemia identify high-risk LDL cholesterol levels, which are used as the basis for treatment decisions in the NCEP guidelines. The available evidence suggests that total cholesterol levels are insufficient in identifying youth with elevated LDL cholesterol levels with sensitivities in the order of 50% when a total cholesterol level above 5.2 mmol/L (200 mg/dL) is applied as per the NCEP guidelines.[271, 278, 279]

In the most recent guidelines from the AAP,[220] additional triggers for lipid and lipoprotein screening are recommended, particularly for overweight or obese youth. This modification is in reply to the rapid increase in the prevalence of overweight and obesity in the US and other industrialised nations since the 1980s,[227-229, 280] and the associated evidence for the consequences of the childhood *obesity epidemic* that have accumulated in this time. As outlined earlier, lipid and lipoprotein abnormalities have been shown to be common in overweight and obese children,[281-284] with low HDL cholesterol and high triglyceride levels particularly prevalent. The clustering of metabolic abnormalities such as insulin resistance and other factors associated with the metabolic syndrome in overweight and obese adolescents is also cause for concern. For example, Sinaiko et al.[285] found evidence of a significant interaction between obesity and insulin resistance and their effect on CVD risk factors, including HDL cholesterol and triglycerides but not LDL cholesterol, in a cross-sectional sample of adolescents. Therefore, the *obesity-associated* dyslipidemia is distinctly different to the more traditional elevation of total or LDL cholesterol levels associated with family history of premature CVD or hypercholesteremia. A number of cross-sectional studies have consistently shown increased adiposity to be the most effective predictor of dyslipidemia compared with other factors assessed, including family history of premature CVD and hypercholesterolemia.[149, 282, 286-299] Adding further credence to these data, longitudinal findings from the Cardiovascular Risk in Young Finns and Bogalusa Heart

Study have also shown adiposity at baseline, as well as change in adiposity, to be the best predictors of follow-up blood lipid and lipoprotein levels outside of the baseline lipid measurement.[161, 231] Combining these findings with those covered in section 1.5.4 that demonstrated the efficacy of weight reduction in improving lipid and lipoprotein levels in youth, it becomes evident why the revised guidelines included overweight or obese youth for lipid screening. Nevertheless, the diagnostic utility of screening overweight or obese youth for the identification of either concurrent or future lipid abnormalities have not been formerly assessed.[199] An equally important consideration in the diagnostic utility of blood lipid and lipoprotein screening in youth is the cut-points used to stratify risk.

### 1.6.3 CUT-POINTS

Dichotomous cut-points for risk stratification aim to define those who are at an increased risk of developing clinically significant CVD, and in whom risk modification will reduce the likelihood of subsequent complications. The initial NCEP report published a single set of fixed cut-points that defined borderline-, and high-risk cut-points for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides at values coinciding with the 75$^{th}$, and 95$^{th}$ percentiles (25$^{th}$, and 5$^{th}$ for HDL cholesterol) of the entire population distribution from the LRC Prevalence Study.[196, 210] The LRC Prevalence Study was a multi-stage NHLBI survey of lipid and lipoprotein levels and other CVD risk factors in nine US and one Canadian community conducted between 1972 and 1976.[210] The NCEP cut-points apply to all children and adolescents aged 2 to 19 years, hence do not account for changes in distributions that occur with growth and maturation, or by sex or race[300-302] – as was outlined in section 1.5.5. These cut-points were later adopted in primary prevention guidelines from the AHA[248] and AAP[247] as the basis for identifying children and adolescents at high-risk of CVD.

A report from Morrison[303] called for the NCEP cut-points to account for pubertal stage, age, sex, and race after demonstrating marked differences in the prevalence of NCEP defined borderline- and high-risk LDL cholesterol in these groups in cross-sectional LRC data. The report by Friedman et al.[276] highlighted in the previous section, showed that the utility of prediction of adult dyslipidemia using the NCEP cut-points differed according to baseline age. For example, the lowest sensitivity occurred at ages 11 to 16 years, while specificity was lowest at ages 11 and 13 years; the years that coincide with puberty. Analyses by sex- or race- were not possible due to low stratum size. Collectively, these data provided

the first diagnostic evidence of the limitations of fixed cut-points for all children and adolescents.

In an attempt to account for age and sex in the interpretation of fasting lipid and lipoprotein profiles, Jolliffe and Janssen[251] recently proposed new lipid and lipoprotein cut-points for adolescents aged 12 to 19 years based on combined data from three National Health and Nutrition Examination Surveys (NHANES). These cut-points were derived using age- and sex- specific growth curves that were linked to adverse adult lipoprotein thresholds established by the NCEP adult treatment panel.[304] This brought lipid and lipoprotein risk definitions in line with age- and sex-specific percentile distributions already established to define risk presence for both hypertension[305] and overweight and obesity.[306, 307] In an editorial to the Jolliffe and Janssen paper, Gidding[252] outlined limitations of using NHANES data in defining cut-points that included: (1) a substantial proportion of the NHANES cohort was excluded from lipid and lipoprotein analysis because of non-fasting status at presentation; and (2) only single lipid and lipoprotein measurements were collected in the NHANES cohort thus the cut-points do not take into account measurement variability of lipid and lipoprotein levels that have been documented.[161, 308-310]

As it is unknown whether the new cut-points provide an improved prediction of those adolescents most likely to develop CVD risk later in life, one of the primary aims of this thesis is to compare the utility of the fixed cut-points stipulated by NCEP and those based on distributions from NHANES suggested by Jolliffe and Janssen to predict both dyslipidemia in adulthood and markers of preclinical atherosclerosis in adulthood.

It is also necessary to note that the 2008 update of lipid screening guidelines from the AAP also provided age- and sex-specific cut-points. However, the authors did not mention the work by Jolliffe and Janssen in their discussion and instead, proposed yet another, untested, set of cut-points for classification based on percentiles from the LRC Prevalence Study.[311] The proposed new cut-points had a number of limitations that were not properly considered and have been the subject of a letter to the editor by the author of this thesis (original contribution I). For example, the proposed lipid and lipoprotein distributions were derived from Caucasian children in the LRC study[311] and as such, should be applied to biracial populations with caution. Moreover, although the authors recommend lipid screening to commence from the age of two years, the proposed cut-points presented began from age five; hence, it was not clear how blood lipid and lipoprotein concentrations of children aged

from two to four years should be classified. Because of the recency of these *revised* cut-points, they were not examined in this thesis.

## 1.7. AIMS OF THIS THESIS

This thesis makes use of data from three population-based prospective cohort studies conducted in Australia (the Childhood Determinants of Adult Health, CDAH, Study), Finland (the Cardiovascular Risk in Young Finns Study), and the United States (the Bogalusa Heart Study). These studies collected baseline blood lipid and lipoprotein measures in children and adolescents in the 1980s, with follow-up measures collected 15-20 years later when participants were young adults. The specific aims examined for this thesis make use of these data, and are outlined below:

1. To examine in the CDAH study:
    a. the long-term (20 year) tracking of blood lipid and lipoprotein levels from childhood and adolescence to young adulthood (Chapter 3); and
    b. factors that affect the tracking of blood lipid and lipoprotein levels from childhood to adulthood (Chapter 3).

2. To determine the utility of two classifications (NCEP vs. NHANES) of paediatric dyslipidemia to predict dyslipidemia in adulthood (Chapter 4).

3. To determine the utility of paediatric LDL cholesterol and HDL cholesterol dyslipidemia classifications (NCEP vs. NHANES) in predicting carotid artery IMT in adulthood (Chapter 5).

4. To assess whether maintaining or changing LDL cholesterol and HDL cholesterol dyslipidemia status from adolescence to adulthood has an effect on carotid artery IMT measured in adulthood (Chapter 5).

## 1.8. SUMMARY

The converged evidence from studies reviewed in the preceding text provides support for: the origin of atherosclerosis in early life; the association of risk factors in youth with early signs of atherosclerosis; and the prediction of adult risk status from childhood or adolescent risk factor levels. Because of this evidence, guidelines for the screening and management of lipid disorders in children and adolescents were established. In recent times, reports from the US Preventive Service Task Force,[250] the American Heart Association,[249] and others,[220, 251-253] have outlined challenges with the existing guidelines and have called for their revision.[196] One area of the existing guidelines outlined for revision includes the lipid and lipoprotein cut-points used to assign risk status. Two classifications of paediatric dyslipidemia have been circulated that attempt to define normal-, borderline-, and high-risk lipid blood levels. Nevertheless, no study has assessed which of these classifications is most effective for predicting those who will develop dyslipidemia or high preclinical atherosclerosis (carotid IMT) in adulthood. A comparison of the predictiveness of these classifications may provide a more accurate assessment of those children and adolescents at high-risk for CVD later in life and help identify those who may benefit most from intervention. The principal aim of this study was therefore to examine the utility of paediatric dyslipidemia classifications that define normal-, borderline-, and high-risk lipid and lipoprotein levels to predict dyslipidemia and high carotid IMT in adulthood. A number of related aims were to examine tracking of blood lipid levels from childhood to adulthood; factors that affect tracking of blood lipid levels from childhood to adulthood; and whether changes in blood lipid levels between adolescence and adulthood had an effect on the level of arterial thickening measured in adulthood.

# 2. METHODS

## 2.1. INTRODUCTION

This chapter describes the methods employed in this study. The primary data sources utilised to examine the aims of this study include: The Childhood Determinants of Adult Health (CDAH) Study, The Cardiovascular Risk in Young Finns Study, and the Bogalusa Heart Study. This Chapter provides an overview of the CDAH, Young Finns, and Bogalusa studies and describes in-depth, the methodological aspects central to this thesis. Extended methods of the CDAH study is included, as the follow-up has not previously been described in detail. Detailed descriptions of some measures that are not used in pooled analyses between the three cohort studies are described in the relevant results chapters where they are used.

## 2.2. AUSTRALIAN DATA: THE CHILDHOOD DETERMINANTS OF ADULT HEALTH (CDAH) STUDY

The 2004-2006 CDAH study is a population based, prospective follow-up of the 1985 Australian Schools Health and Fitness Survey (ASHFS) that collected extensive lifestyle and biological measures on a representative sample of 8498 Australian school children aged 7 to 15 years. The CDAH study was established to examine childhood predictors of adult CVD and diabetes with long-term follow-up of the cohort over the coming decades.

### 2.2.1 SAMPLE SELECTION AND PARTICIPANTS

The sampling procedures of the original ASHFS have been described in detail elsewhere,[149, 312] but will be summarised here. The sampling frame was defined as all students aged 7 to 15 years who were currently attending schools (primary or secondary, government, catholic, or independent) in Australia. Children were selected using a two-stage (school, then student) probability plan that was designed to yield a self-weighted sample (that is, the data do not need to be weighted since the sample design gives each individual an equal chance of being selected). The first stage of sampling selected schools with probability proportional to enrolment numbers. Of the 121 schools selected, 90.1% (N = 109) agreed to participate. The distribution of schools surveyed is displayed in Figure 11. The second stage utilised simple random sampling to ascertain children within each age and sex category from the selected

schools. Of the 12,578 students that were invited to participate in the study, 8498 participated representing an overall response proportion of 67.5%.

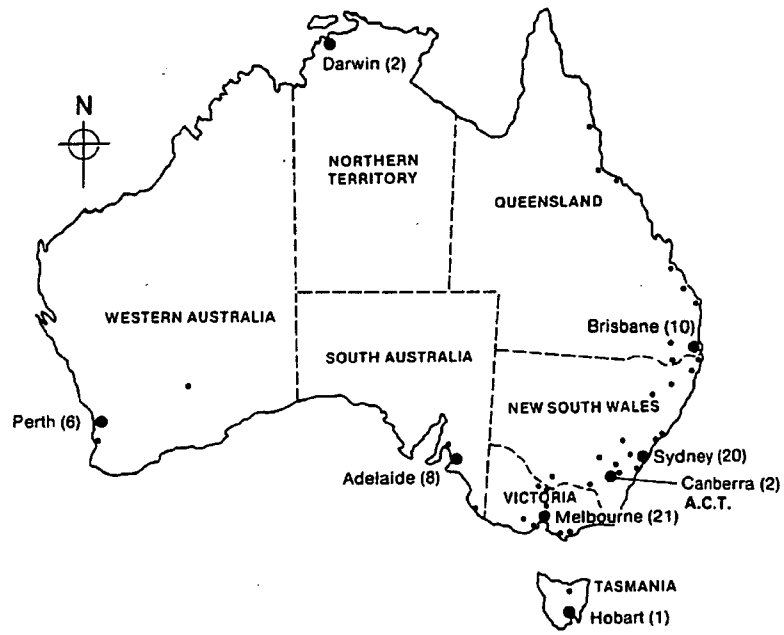


**Figure 11.** Distribution of schools surveyed in 1985 ASHFS.

Between 2001 and 2004, 6840 (81%) of the original participants were traced using the following methods: the Australian National Death Index, current and historical electoral rolls, telephone listings, and school and family networks. Eighty-six participants were deceased at the time of follow-up. The main causes of death were: injury and poisoning (includes suicide, 39 cases), accidents (8 cases), neoplasm (5 cases), heart and circulatory diseases (4 cases). Of the remainder of traced participants, 5170 (61% of the original cohort) contributed data to the follow-up with 2410 (28% of the original cohort) attending one of 34 field-clinics held Australia wide between May 2004 to May 2006. Figure 12 displays participation, response proportions, and loss to follow-up in the ASHFS (1985) and CDAH (2004-2006) studies.

At baseline, consent from both parent and child was required for inclusion in the study; at follow-up, all participants gave written informed consent. The baseline study was approved by the State Directors General of Education and the follow-up survey was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee.

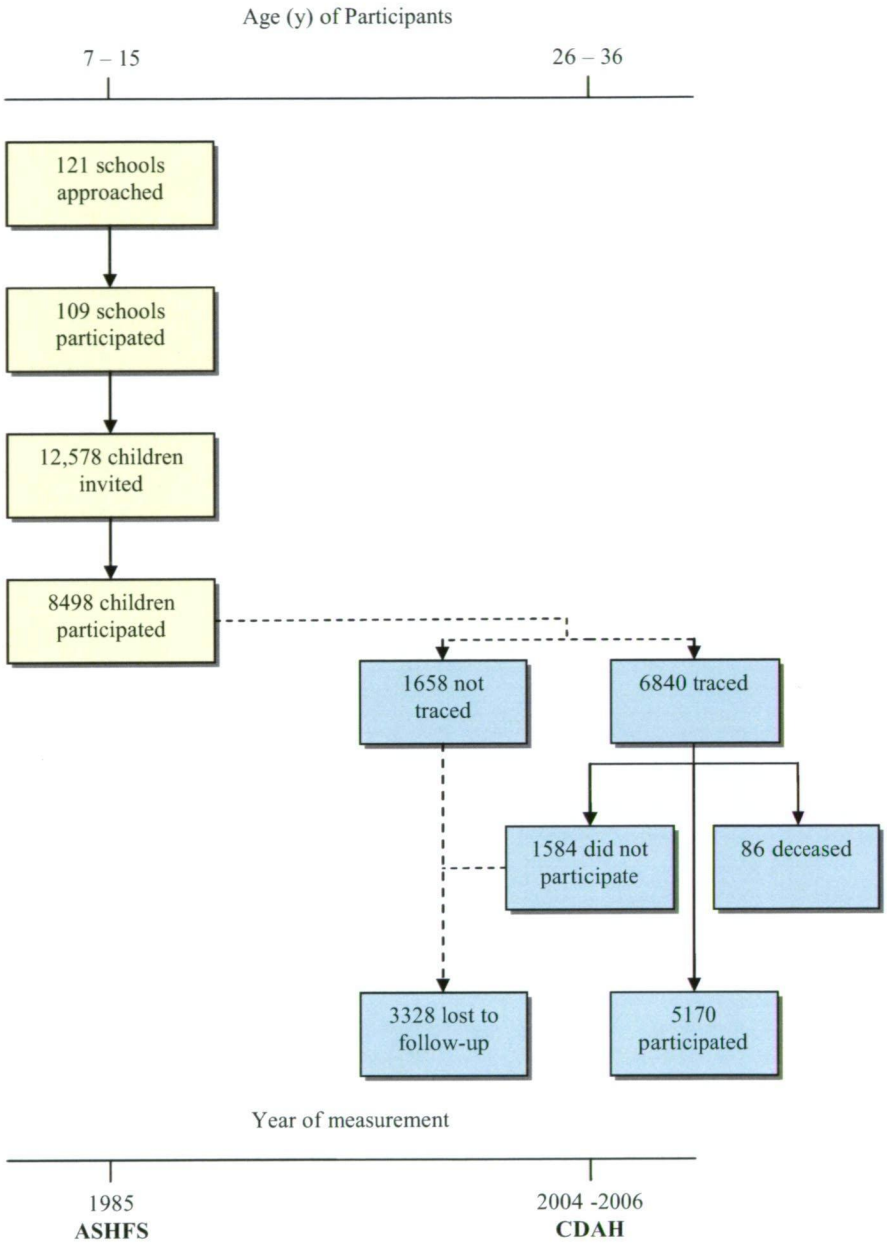


**Figure 12.** Participants and response proportions in ASHFS; and participants, response proportions and loss to follow-up in the CDAH study. Note. Of the 1584 that did not participate, 817 of these did not respond to attempts to contact and 767 of these refused to participate in the study.

## 2.2.2 DATA COLLECTION

*Baseline (1985)*

A team consisting of 10 data collectors and a supervisor were recruited in each state and territory to administer the questionnaire and physical examinations. Blood samples were collected by qualified nurses with experience in venipuncture. All personnel underwent a period of training in the method of standardised protocols prior to the commencement of data collection. Personnel were allocated specific tasks to ensure a minimum amount of people were involved in collecting specific measurements throughout the survey, thus limiting the potential of inter-measurer error. Registered site visits were undertaken by the principal investigators to maintain protocol standardisation throughout the survey.

Field clinics were used as the primary source of data collection in 1985. Clinics were conducted at the participating school over consecutive school days. A range of measurements were collected during clinics, including: *field tests* (height, weight, girths, field-based fitness), *technical tests* (muscular strength, skin folds, blood pressure, lung function, sub-maximal fitness), *laboratory-based tests* (maximal fitness, body density), fasting blood samples (lipids and lipoproteins), and questionnaire (diet, physical activity, smoking, alcohol and general health). Field tests were completed by all participants. Technical tests and blood samples were collected on those aged 9, 12, and 15 years. Of this sub-sample of 9, 12, and 15 year olds, 1919 participants of the total eligible 2809 (68.3% response proportion) consented to provide blood samples. Laboratory-based tests were conducted on a sub-sample of those aged 9, 12, and 15 years (N=290). Participants aged 9 to 15 years completed questionnaires. The level of participation for each test category is displayed in Table 4. A strict testing order was adhered to, with anthropometric measures performed first, followed by fitness tests after a thorough warm-up. Questionnaires were then administered to children in groups of four with supervision provided by data collectors. Participants aged 9, 12, and 15 years attended the following day for the completion of technical tests. Fasting blood samples were collected at the school as soon as possible after the field and technical data collection had been completed.

**Table 4.** Response Proportions and Number of Participants in 1985 ASHFS*

| Sex & Age | Field & Technical† | | | Questionnaire | | | Blood Sample | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | % | Response proportion (%) | N | % | Response proportion (%) | N | % | Response proportion (%) |
| BOYS | | | | | | | | | |
| 7 | 475 | 11.0 | 71.5 | | | | | | |
| 8 | 490 | 11.4 | 75.0 | | | | | | |
| 9 | 482 | 11.2 | 71.1 | 490 | 14.6 | 71.9 | 371 | 36.7 | 77.0 |
| 10 | 492 | 11.4 | 77.0 | 493 | 14.7 | 75.7 | | | |
| 11 | 489 | 11.4 | 68.9 | 482 | 14.4 | 66.6 | | | |
| 12 | 494 | 11.5 | 66.0 | 486 | 14.5 | 63.4 | 349 | 34.5 | 70.6 |
| 13 | 465 | 10.8 | 65.4 | 468 | 14.0 | 65.0 | | | |
| 14 | 467 | 10.9 | 65.5 | 472 | 14.1 | 66.5 | | | |
| 15 | 450 | 10.5 | 63.3 | 461 | 13.8 | 64.8 | 291 | 28.8 | 64.7 |
| Subtotal | 4304 | 100 | 69.3 | 3352 | 100 | 67.7 | 1011 | 100 | 70.8 |
| GIRLS | | | | | | | | | |
| 7 | 478 | 11.4 | 69.6 | | | | | | |
| 8 | 496 | 11.9 | 73.2 | | | | | | |
| 9 | 487 | 11.7 | 72.5 | 492 | 15.2 | 73.8 | 348 | 38.3 | 71.5 |
| 10 | 497 | 11.9 | 75.2 | 496 | 15.3 | 73.7 | | | |
| 11 | 483 | 11.6 | 70.5 | 483 | 14.9 | 69.5 | | | |
| 12 | 489 | 11.7 | 65.7 | 488 | 15.1 | 64.7 | 307 | 33.8 | 78.9 |
| 13 | 438 | 10.5 | 58.6 | 437 | 13.5 | 59.1 | | | |
| 14 | 405 | 9.7 | 53.6 | 417 | 12.9 | 55.3 | | | |
| 15 | 407 | 9.7 | 56.4 | 421 | 13.0 | 58.5 | 253 | 27.9 | 62.2 |
| Subtotal | 4180 | 100 | 66.1 | 3234 | 100 | 65.0 | 908 | 100 | 70.9 |
| **TOTAL** | **8484** | | **67.5** | **6586** | | **66.1** | **1919** | | **68.3‡** |

* This table was reproduced from the original ASHFS report,[312] which included data for 8484 participants; however, data from a further 14 participants were identified in the dataset when follow-up was initiated and were included in the analyses.

† Field and technical tests included height, weight, girths, sit and reach, sit-ups, standing long jump, push-ups, 50m sprint, and 1.6km sprint.

‡ The original published table had the incorrect response proportion for blood sample measures and has been rectified here (incorrect 70.8% vs. actual 68.3%).

*Follow-up (2004-2006)*

Field clinics were again established as the primary avenue for data collection at follow-up. Participants unable to attend a clinic were offered alternate options to provide data to the follow-up. Consequently, the levels of participation (in terms of data obtained) differed considerably for participants in the follow-up survey, and are displayed in Table 5. Measures collected at follow-up included: blood biochemistry, physical measurements (blood pressure,

anthropometry, lung function, cardiorespiratory fitness, muscular fitness, bone density, carotid and brachial artery ultrasound studies), and questionnaire data (diet, physical activity, smoking habits, alcohol use, general health, personal and family medical history, socioeconomic status, reproductive health, and mental health). Participants were mailed self-complete questionnaires one to two weeks before their scheduled clinic visit and were asked to bring the completed forms with them to the clinic.

**Table 5.** Levels of participation in the 2004-2006 CDAH study

| | Males | | Females | | All | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Clinic | 1150 | 47.2 | 1260 | 46.1 | 2410 | 46.6 |
| Enrolment data only* | 620 | 25.4 | 604 | 22.1 | 1224 | 23.7 |
| Short questionnaire (no clinic attendance)† | 445 | 18.3 | 464 | 17.0 | 909 | 17.6 |
| Full questionnaire (no clinic attendance)‡ | 159 | 6.5 | 278 | 10.2 | 437 | 8.5 |
| Remote pathology (no clinic attendance) | 62 | 2.5 | 122 | 4.5 | 184 | 3.6 |
| Other | 2 | 0.1 | 4 | 0.1 | 6 | 0.1 |
| Total | 2438 | 100 | 2732 | 100 | 5170 | 100 |

*All participants completed the enrolment questionnaire; these values represent those participants from whom no other data were collected. The enrolment questionnaire was self-completed and returned by mail or completed by telephone interview when follow-up contact with participants was first made between 2001 and 2004. The short or long questionnaire was administered to those that indicated at time of enrolment (2001-2004) their willingness to participate but who were unable to attend clinics when re-contacted (2004-2006). The enrolment questionnaire is provided in Appendix 2.

† The short questionnaire is provided in Appendix 3.

‡ The full questionnaire is provided in Appendix 4

Percentages may not add to 100 because of rounding.

Data collection teams for each state consisted of eight locally recruited staff and three core staff. The core staff (project manager, trained phlebotomist, ultrasound technician) was maintained throughout fieldwork clinics. Staff recruited locally were briefed on the background of the survey before undertaking training workshops to ensure measures were collected according to standard procedures of the survey. A central group of technicians travelled to each state from the Menzies Research Institute to conduct the training workshops. Personnel were trained to perform specific tasks, with compliance to standardised protocols monitored throughout data collection by the project manager.

Geographic Information Systems software was used to map participants' current postcode (Figure 13) to identify local clinic sites that would be accessible to as many participants as possible. Clinic locations were selected to maximise the proportion of enrolled participants living within a 10 kilometre radius (55% of participants residing within this 10km radius of the site ultimately attended clinics). Clinic venues included community halls, sport halls, church halls, and schools. The field clinics were conducted between 7:00 am and 2:00 pm on weekdays and weekend days. Approximately 15 to 30 participants attended daily. Participants moved through the clinic in a circuit-like manner that took 2.5 to 3 hours to complete. The order of measurements followed a strict protocol. Anthropometric, blood pressure, vascular ultrasound examinations, and blood samples were collected before participants were allowed breakfast. Following breakfast, participants completed a computer-administered mental-health questionnaire, lung function test, cardiorespiratory fitness test, strength tests, ultrasound assessment of bone density, and were issued a pedometer.

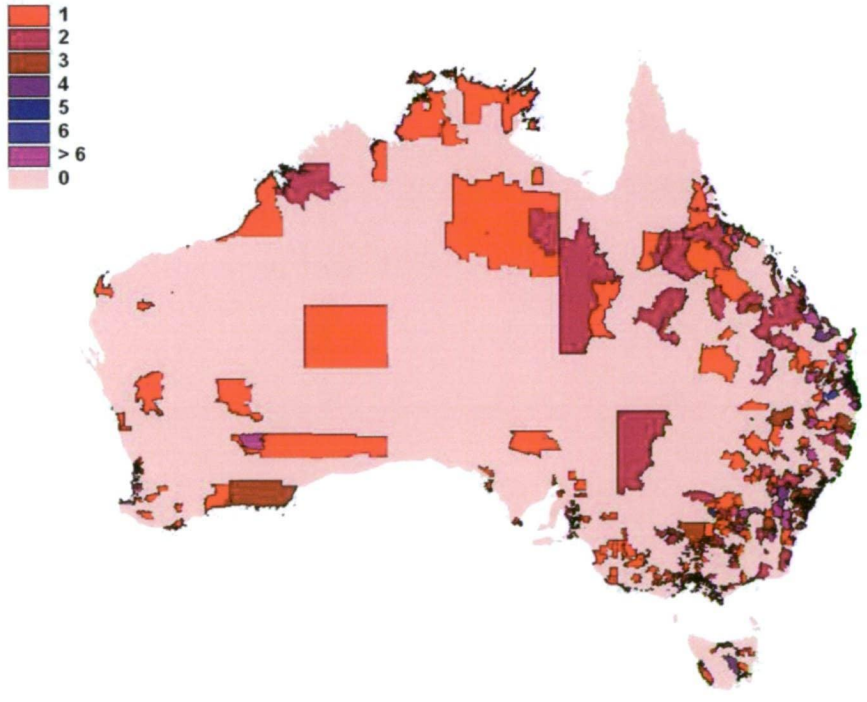


**Figure 13.** Distribution of CDAH participants according to postcode as of January 2004.

## 2.2.3 MEASURES

The baseline and follow-up measures relevant to the aims of this thesis are described in detail below. A full description of other tests and protocols from the baseline survey can be found in the ASHFS handbook.[312]

### 2.2.3.1 Blood collection and biochemistry

The LRC procedures[313] were followed for blood sampling in 1985, with exceptions noted below. Approximately 10 ml of venous blood was drawn from the antecubital vein of seated participants after a 12-hour fast. Solid Ethyl Diamine Tetra-Acetic Acid was used as the anticoagulant. One minor departure from the LRC procedure concerned the initial cooling of blood samples before specimen processing. The LRC states that blood samples should be cooled immediately on wet ice, but in some centres this was done by placing them in a domestic refrigerator. Plasma was separated from blood cells by centrifugation and then stored at approximately 4°C until transport to the Central Analytical Laboratory at the Flinders Medical Centre (Adelaide, South Australia). On a weekly basis, samples were flown from the nearest state capital in insulated containers and reached the central laboratory cold but not frozen on the day of dispatch. Throughout the baseline survey, the analytical laboratory met the criteria for precision accuracy as specified for standardisation by the WHO Collaborating Centre for Reference and Research in Blood Lipids (Centers for Disease Control and Prevention, Atlanta, Georgia). Samples with unknown assigned values from the Centre were incorporated into each run. Plasma total cholesterol and triglycerides were determined using a Technicon Auto Analyzer II (Technicon Instrument Corp, Tarrytown, New York, USA), HDL cholesterol was analysed following precipitation of apo-B containing lipoproteins with heparin manganese. The concentration of LDL cholesterol was determined indirectly using the Friedewald formula:[314] LDL cholesterol = total cholesterol - (triglycerides/2.2 + HDL cholesterol)

At follow-up, 30 mL of venous blood was drawn from the antecubital vein of reclined (seated or lying) participants after an overnight fast of at least eight hours. Samples for lipid assays were stored in white-top serum tubes and allowed to clot (coagulate) for 15 minutes at room temperature prior to centrifugation. All samples were centrifuged within two-hours of venipuncture and then stored upright in a fridge or ice container and held at approximately 4°C. At the completion of each clinic day, all samples were sealed in an

insulated container with cold packs, and dispatched via overnight courier to the central analysing laboratory (MedVet, Adelaide, South Australia) where assays were completed. For 12 clinic days (once per clinic location in the states of New South Wales and Western Australia, as well as one clinic site in Victoria and Queensland), temperature data loggers where included in the insulated containers dispatched for overnight delivery. Temperature during transport ranged from 0.7° to 3.6°C (mean temperature, 2.6°C). Serum total cholesterol, triglyceride, and HDL cholesterol concentrations were determined enzymatically using an Olympus AU5400 automated analyser (Olympus Optical, Tokyo, Japan). LDL cholesterol was calculated using the Friedewald formula.[314]

Throughout both the baseline (1985) and follow-up (2004-2006) period, the analytical laboratory met the criteria for precision accuracy as specified for standardisation by the WHO Centre for Reference and Research in Blood Lipids (Centers for Disease Control and Prevention, Atlanta, Georgia). Samples with unknown assigned values were incorporated into each analytical run or immediately following calibration of the analyser. Over the course of the 2004-2006 study, inter-assay coefficient of variation ranged from 2.1 to 2.8% for total cholesterol, 4.2 to 5.4% for HDL cholesterol, and 3.0 to 4.6% for triglycerides. Also during follow-up, duplicate aliquots from a single blood draw were collected from the first participant scheduled for each clinic day (N=108) to examine measurement errors associated with collection, processing, and analysis of blood samples. All duplicate assays were conducted by technicians blinded to the first-run results. The coefficients of variation were 1.6% for total cholesterol, 2.8% for HDL cholesterol, and 2.6% for triglycerides.

#### 2.2.3.2 Blood pressure

Blood pressure measurements were obtained from the left brachial artery using a standard mercury sphygmomanometer at baseline, and from the right brachial artery using a digital automatic monitor (Omron HEM907, Omron Healthcare Inc, Kyoto, Japan) at follow-up. All measurements were taken after the participant had been seated with legs uncrossed for five minutes. The participant's upper arm girth was measured and an appropriate cuff size was selected. At baseline, systolic pressure was recorded at Korotokoff's first phase and diastolic pressure was recorded at both Korotokoff's fourth and fifth phases. Korotokoff's fifth phase was taken to represent diastolic pressure. This procedure was repeated after five minutes, and the mean of the two measurements was used in the analyses. At follow-up, three consecutive measurements of systolic and diastolic pressures were recorded. Each consecutive

measurement was separated by a one minute interval to avoid any spasms in the artery that could lead to erroneous measurements. The mean of these three measurements was used in the analyses.

### 2.2.3.3 Anthropometry

Anthropometric measurements of height and weight were collected at baseline and follow-up. Standing height was measured to the nearest 0.1 cm using a portable stadiometer, with the participant in bare feet and any obstructive headwear removed. Weight was measured with participants in light clothes without shoes using regularly calibrated bathroom scales that recorded to the nearest 0.5 kg at baseline, and with a digital Heine portable scale to the nearest 0.1 kg at follow-up. Body mass index at baseline and follow-up was calculated using the formula: BMI = weight (kg) / [height (m)]$^2$.

### 2.2.3.4 Smoking

At baseline, participants aged 9 years and older completed a questionnaire that included the following question: 'How long have you been smoking regularly? (regularly means 1 or more times a week)'. Children and adolescents could respond: 'I don't smoke'; 'just started'; '1 to 6 months'; '7 months to 1 year'; '1 to 2 years'; '2 to 4 years'; 'more than 4 years'. The responses were collapsed into a binomial categorical variable defining current smoking status as: 0=smoking less than once per week (<1/week), i.e. those that indicated 'I don't smoke', and 1=smoking at least once per week (≥1/week), i.e. those that indicated any of options 2 to 7. At follow-up, participants were asked: 'Over your lifetime, have you smoked at least 100 cigarettes, or a similar amount of tobacco?' Those who indicated they had smoked at least 100 cigarettes or equivalent tobacco over their lifetime were then asked the following question: 'How often do you <u>now</u> smoke cigarettes, cigars, pipes or any other tobacco products?': Participants could respond: 'Daily (≥1/day)', 'At least once a week (but not daily) (≥1/week)', 'Less often than weekly (<1/week)', 'Not at all (ex-smoker)'. At follow-up, participants were classified as current smokers if they indicated cigarette smoking on a weekly basis or more often, i.e. those who had smoked at least 100 cigarettes over their lifetime and indicated they were 'Daily', or 'At least once a week' smokers. Those that indicated they had not smoked at least 100 cigarettes in their lifetime, or indicated they

smoked less often than weekly or were ex-smokers were considered as not current smokers at follow-up.

For the analyses, these baseline and follow-up definitions of current smoking status were used as they provided the most consistent definition across the three cohorts. A summary of the smoking information collected in each study and reasoning for the choice of the smoking definition is provided in Appendix 5.

#### 2.2.3.5 Carotid artery ultrasound studies

Epidemiological studies that perform ultrasonography typically have participants attend specialist clinics for scans in addition to any clinic attendance that involves physical measurements. Because a number of the 34 field clinic locations in the CDAH study did not have access to all of the necessary resources to perform ultrasound scans (including specialist scanning facilities, equipment, or personnel); all ultrasound studies were performed at the field site. This had additional benefits that included reduced participant burden and the use of a single machine to perform all ultrasound studies.

B-mode ultrasound studies of the carotid arteries were performed using a portable Acuson Cypress (Siemens Medical Solutions USA Inc., Mountainview, CA, USA) ultrasound machine with a 7.0-MHz linear-array transducer by a single technician who travelled to each of the field clinics. The Cypress is a miniaturised cardiac and vascular ultrasound system that is more portable than standard clinic-based machines owing to the incorporation of an in-built foldable monitor, removable transducers, the omission of a recording device, and the subsequent low weight (8 kg) of the complete system (Figure 14). Before its inclusion in CDAH, a study was conducted to determine if pre-clinical measures of vascular structure and function derived from the Cypress ultrasound machine were comparable to those from a clinic-based machine (Acuson Sequoia 512, Siemens Medical Solutions USA Inc., Mountainview, CA, USA) used routinely in clinical practice (original contribution II). This a-priori validation work was necessary to provide confidence in the measurements obtained from the machine (measures that have ultimately been used as outcomes in this thesis), and because data pooling with the Cardiovascular Risk in Young Finns Study, which had used the Acuson Sequoia 512 machine to perform carotid artery studies, was anticipated. Briefly, consecutive carotid and brachial artery images were recorded from a convenience sample of 23 apparently healthy young adults (mean age 31 years) with the portable type and clinic type instruments. The analyses revealed a high level

of agreement between the two machines for measurements of maximum (mean difference = 0.001 mm) and mean carotid IMT (mean difference = -0.025 mm), and brachial flow mediated dilatation (mean difference = 0.27 %). It was concluded that measurements of vascular structure and function derived from the portable machine compared well with those from a clinic based machine.[315]

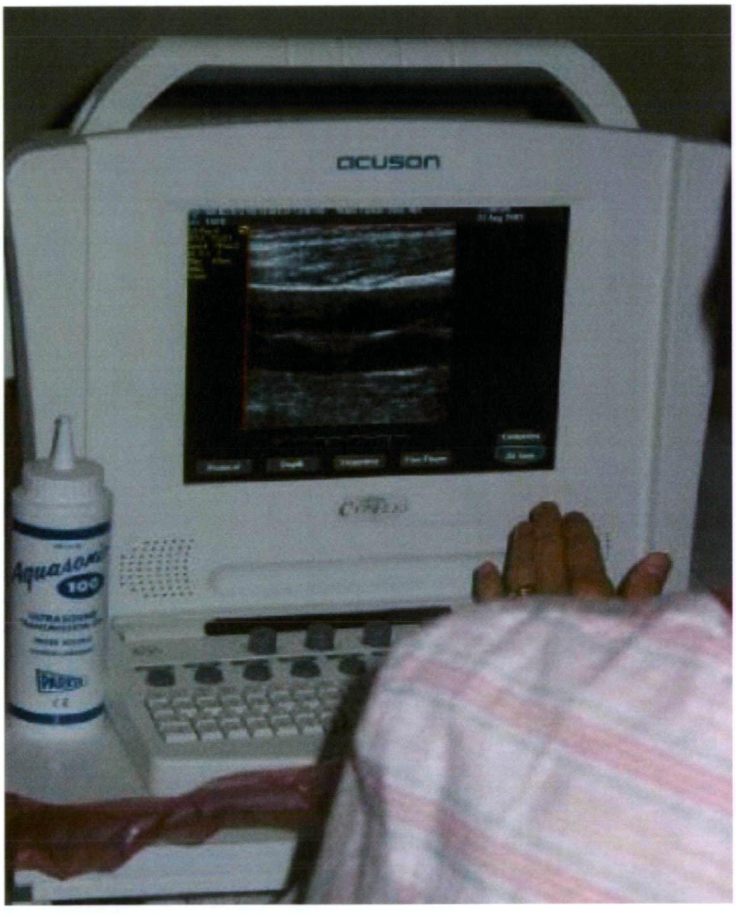


**Figure 14.** Acuson Cypress portable ultrasound machine

Where facilities allowed, a room separated from other testing stations was used for ultrasound examinations at field-clinic sites. Difficulties in performing ultrasound examinations in a field-work setting were recorded by the technician and are listed in Table 6. Temperature extremes (cold or heat) were identified as an issue for both participant and technician comfort during ultrasound examination at some clinics. Blankets as well as portable heaters and fans were used in an attempt to maximise participant comfort. Air temperature was recorded for each participant at the start of the ultrasound examination.

Mean ± standard deviation (SD) temperature was 21.0±3.0°C when carotid artery examinations were performed.

**Table 6.** Problems encountered in performing ultrasound examinations in a field-work setting

| Problem encountered | Frequency | Approximate number of participants not measured as a consequence of problem encountered |
|---|---|---|
| -Equipment did not arrive from transport company in time for clinic | 1 clinic (4 clinic days) | 37* |
| -Excessive light that made visualisation of the digital monitor difficult | 1 clinic (3 clinic days) | 0 |
| -Excessive noise, affected concentration: | | |
| -renovations and construction work to adjacent building | 2 clinic days | 0 |
| -noise from other field-clinic stations | 2 occurrences | 0 |
| -Power failures | 3 occurrences | 1 |
| -Participant and technician comfort | | |
| cold (insufficient heating) | Several | 0 |
| heat (insufficient cooling) | Several | 0 |
| -Probe error | 3 occurrences | 0 |
| -Software or technician error | | |
| application error | 6 occurrences | 2 |
| transfer error | 2 occurrences | 2 |
| accidental eraser | 2 occurrences | 2 |

*A total of 48 participants were affected by the failure of equipment to arrive in time for this clinic, however, 11 participants returned for measurement at a subsequent clinic.

The ultrasound technician was trained in the carotid artery imaging protocols used in the Cardiovascular Risk in Young Finns study by one of their ultrasonographers.[41] During scanning, time-gain-compensation and depth settings were adjusted to optimise image quality. The left common carotid artery and left carotid bulb were traced using a transverse scan. Once the beginning of the flow divider was localised, the probe was rotated 90°. The longitudinal image was then focused on the posterior (far) wall of the vessel and the scanning angle adjusted so that the greatest distance between the lumen-intima interface and the media-adventitia interface was localised. Several 3-5 second real-time images were recorded that included the beginning of the carotid bulb and approximately 30 mm of the common carotid artery. All images were stored in digital format for off-line analysis in the analysis program Image Pro Plus version 5.02 (Media Cybernetics, Inc., Silver Spring, MD, USA). From the 3 to 5 second real-time images, the two highest-quality end-diastolic frames

(coinciding with the R-wave on a continuously recorded ECG) with the clearest vessel boundaries were selected by the reader for measurement. Each image selected for measurement was given a quality score (1 = excellent, 2 = average, 3 = unacceptable) based on the presence of clear vessel boundaries. 80 % of images were rated excellent, 18.9 % were rated average, and 1.1 % were rated as poor. From each of these images, six measurements of the common carotid far wall were taken approximately 10 mm before the border of the carotid bulb (Figure 15). Maximum carotid IMT was defined as the mean of the maximum IMT measurements from the two selected frames. Although carotid IMT measurements for the entire cohort were performed by two readers, all IMT measurements for participants aged 9, 12, and 15 years who had complete measurements available from the 1985 survey were performed by one-reader (the author). This was done to eliminate inter-reader error in this sub-sample. Carotid measurements taken from this sub-sample are used in the present analyses. Intra-rater reproducibility for replicate max IMT measurements was assessed in a random sample of 30 participants. The average absolute difference and standard deviation was 0.02±0.04 mm.

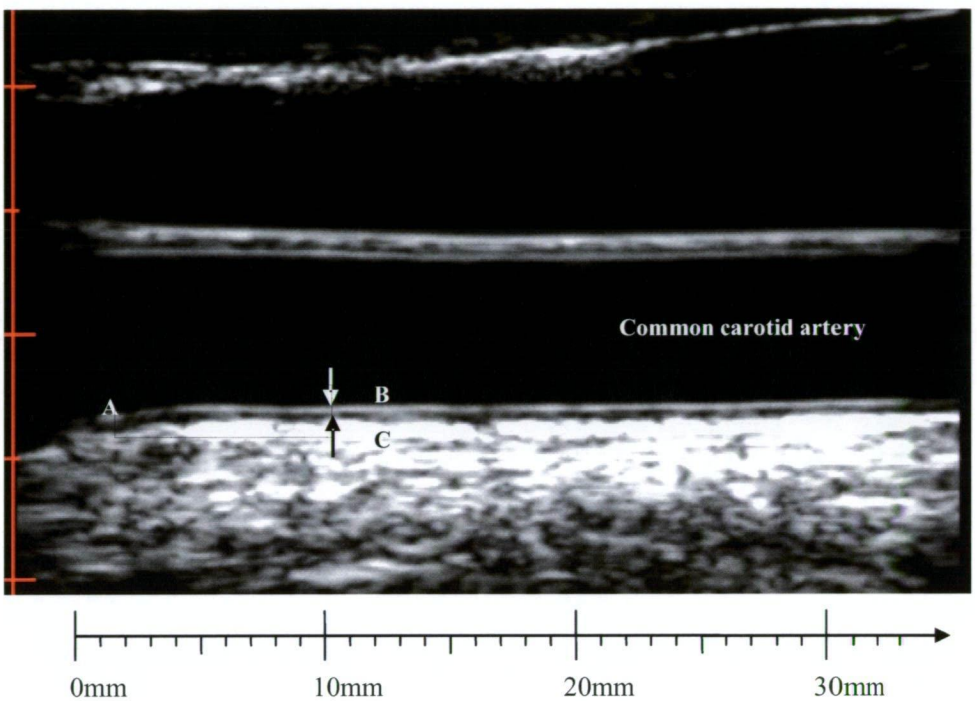


**Figure 15.** B-mode ultrasound image of the left common carotid artery (CCA): A) border of carotid bulb widening (0 mm), B) CCA far wall lumen-intima interface, C) media-adventitia interface. Definitions of the CCA segment in each cohort: CDAH and Young Finns, CCA IMT is taken as the distance between B and C measured in the vicinity 10 mm proximal to the border of the bulb widening (A); Bogalusa, CCA IMT is taken as the distance between B and C measured 0-10 mm proximal to the border of the bulb widening (measurement area highlighted by yellow box).

## 2.3. FINNISH DATA: THE CARDIOVASCULAR RISK IN YOUNG FINNS STUDY

The Cardiovascular Risk in Young Finns Study is an on-going five-centre epidemiologic study of atherosclerosis risk factors and precursors from childhood to adulthood.[316] The study was devised in the late 1970s as a collaborative effort between all university departments of paediatrics or medicine (i.e. in Helsinki, Kuopio, Oulu, Tampere, Turku) to study the levels of CVD risk factors and their determinants in children and adolescents of various ages in different parts of Finland (Figure 16). Two pilot studies in 1978 (N=264, age 8 years)[317] and 1979 (N=634, aged 3, 12 and 17 years)[318] preceded the first main cross-sectional (baseline) study performed in 1980. Thereafter, follow-up studies were conducted at intervals of three years until 1992. The 21-year examination was performed in 2001 when participants were young adults aged 24 to 39 years. It is these data that have been used to study the aims of this thesis. The 27-year follow-up clinics were completed in the first half of 2008.


**Figure 16.** The five centres where data collection for the Cardiovascular Risk in Young Finns Study was conducted.

### 2.3.1 SAMPLE SELECTION AND PARTICIPANTS

The study was carried out in all five Finnish university cities with medical schools and their rural surroundings. In 1980, 4320 children and adolescents aged 3, 6, 9, 12, 15 and 18 years (including equal numbers of males and females) were randomly chosen from the Finnish Social Insurance Institution's national population register of these areas to produce a representative sample of Finnish children. The population register covers the entire population in Finland and is kept up-to-date. The sampling procedure involved assigning a unique personal identification number to girls and boys of each age cohort in each study area. The unique identifying numbers were placed in a random order and every $k^{th}$ girl and every $k^{th}$ boy in each study area was selected so that the sample consisted of the required number of girls and boys. The varying $k$ factors were determined on the basis of sample size and the total number of girls and boys in the different age cohorts in each study area. Letters of invitation to participate were sent to the households of selected children and adolescents. Of those invited, 3596 children and adolescents (83.1% of those invited) participated in the first cross-sectional study in 1980. The 21-year follow-up was performed between September 2001 and January 2002, when 2283 subjects from the original cohort (63 %) were re-examined then aged 24, 27, 30, 33, 36 and 39 years.[316] Data from two time-points were used to examine the aims of this thesis: baseline (childhood and adolescence, 1980) and follow-up (young adulthood, 2001).

The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocols were approved by local ethics committees. Written informed consent was obtained from all participants in 2001 and their parents in 1980.

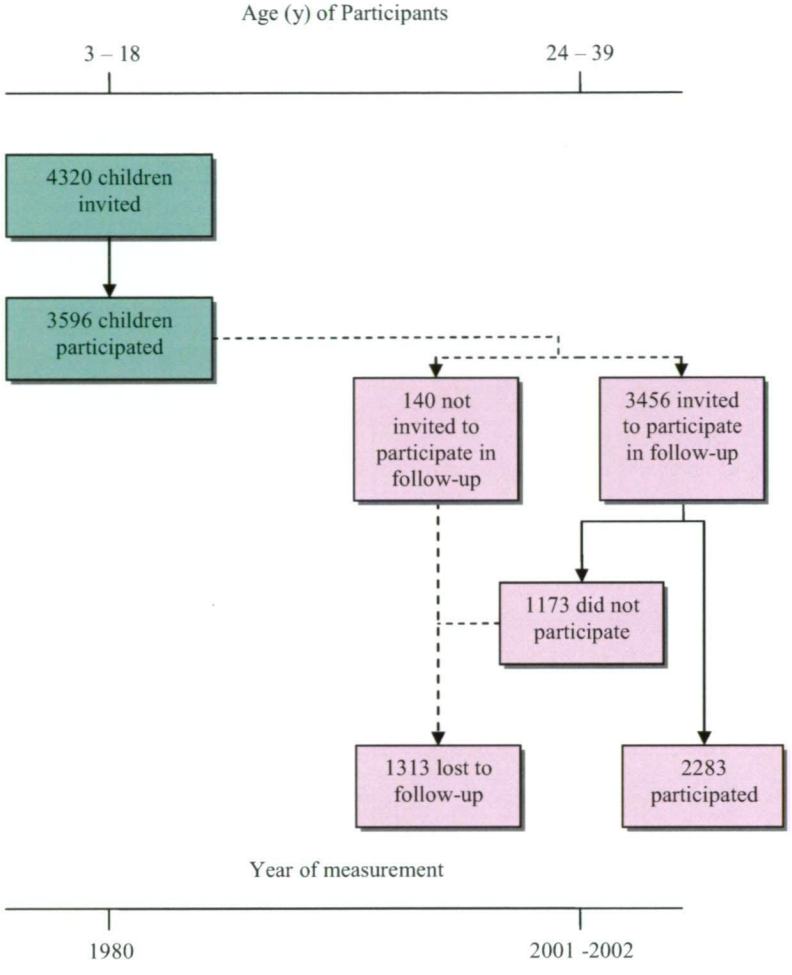


**Figure 17.** Participants and response proportions for the baseline study; and participants, response proportions and loss to follow-up for the 21-year follow-up to the Cardiovascular Risk in Young Finns Study.

## 2.3.2 DATA COLLECTION

*Baseline (1980)*

Prior to physical examination, families were mailed a questionnaire that collected data on socioeconomic status, living environment, health, physical activity, and parent and grand-parent state of health. Physical measurements were performed by trained nurses supervised by a chief investigator of the study. City-dwelling participants were examined at the clinics of

the departments of paediatrics. For rural-dwelling participants, examinations were held at a local health centre (there were 12 rural clinics in total). Participants were examined between 8:00 am and 10:00 am after an overnight fast. A series of measurements were collected at the time of the physical examination, including: anthropometry (height, weight, skin folds, upper-arm girth), directly-measured (by a nurse) pubertal-stage according to the Tanner classification,[319] blood pressure, and venous blood samples (lipids and lipoprotein variables, insulin, glucose). Dietary data were collected on half of the study group (N=1780). Participants aged 12, 15, and 18 years completed a questionnaire on smoking and the use of alcohol.

*Follow-up (2001-2002)*

For the 21 year follow-up, a total of 3456 of the participants in the baseline survey were invited by letter to attend clinics that were scheduled in all five centres (Helsinki, Kuopio, Oulu, Tampere, Turku). Those who did not respond to the invitation letter were later contacted by telephone. Of the 140 not invited, 80 were living abroad, 20 had unknown addresses, 20 had forbidden to be contacted again, and 20 participants in the original cohort were no longer alive. Causes of death for these individuals were suicide (14 cases), motor vehicle crashes (3 cases), alcohol poisoning (1 case), subarachnoid haemorrhage (1 case), and pancreatitis (1 case). Lack of time (33%), absence from the place of residence at the time of examination (13%), and unwillingness to participate (13%) were the main reasons given for non-participation in the follow-up.

Prior to physical examination, participants were mailed a questionnaire that collected data on socioeconomic status, living environment, health, physical activity, diet, smoking, use of alcohol, and parent state of health. Physical measurements were performed by trained nurses supervised by a chief investigator of the study. As per baseline data collection, city-dwelling participants were examined at the clinics of the departments of paediatrics and rural-dwelling participants were examined at one of 12 local health centres. Participants were examined between 8:00 am and 10:00 am after a 12 hour fast. A series of measurements were collected at the time of the physical examination, including: anthropometry, blood pressure, blood samples, and ultrasound studies of the brachial and carotid arteries.

### 2.3.3 MEASURES

All measures from both baseline and follow-up surveys that are relevant to the aims of this thesis are described in detail below. A full description of other measures and protocols from the baseline and follow-up surveys of the Young Finns study can be found elsewhere.[320, 321]

#### 2.3.3.1 Blood collection and biochemistry

All serum lipid determinations were done on fasting samples in duplicate in the same laboratory. In 2001, serum cholesterol and triglyceride concentrations were determined enzymatically (Olympus System Reagent, Olympus Diagnostica, Hamburg, Germany) in a clinical chemistry analyser (AU400, Olympus Optical, Mishima, Japan). HDL cholesterol was analysed after precipitation of very low-density lipoprotein and LDL with dextrane sulphate 500 000.[322] The concentration of LDL cholesterol was calculated using the Friedewald-formula.[314] Details of the methods in earlier studies have been previously published.[217, 323] Due to changes in determination methods and kits during study years, lipoprotein levels from 1980 were corrected to those in 2001 using correction factor equations (this information is detailed in Appendix 6).[218]

#### 2.3.3.2 Blood pressure

Blood pressure was measured using a standard mercury sphygmomanometer at baseline, and using a random zero sphygmomanometer (Hawksley & Sons Ltd, Lancin, UK) at follow-up. All measurements were taken on the right arm, before venipuncture and after the participant had been seated for five minutes. Cuff size was chosen according to arm circumference. Systolic blood pressure was recorded for Korotkoff's first phase. Diastolic blood pressure was recorded at both Korotkoff's fourth and fifth phases. Korotkoff's fifth phase results have been used in the analyses. Readings to the nearest even number of millimetres of mercury were performed three times on each participant. The mean of these three measurements was used in the analyses.

#### 2.3.3.3 Anthropometry

Height and weight measures were taken at both time-points using the following protocols. Height was measured using a wall-mounted Seca stadiometer with 0.5 cm accuracy, and weight was measured in light clothing without shoes with a digital Seca scale to the nearest

0.1 kg. Baseline and follow-up BMI was determined from weight and height measurements recorded in 1980 and 2001 respectively.

#### 2.3.3.4 Smoking

Smoking habits at baseline and follow-up were ascertained as part of a self-administered questionnaire. At baseline, information on smoking habits was collected in conjunction with the medical examination in an isolated room where the participants could respond confidentially and undisturbed. Current smoking habits at baseline and follow-up were indicated as 'once a day or more often', 'at least once a week but not daily', 'less than once a week', 'stopped smoking or do not smoke at present', and 'never smoked'. Participants were classified as smokers at baseline if they indicated regular cigarette smoking on a weekly basis or more often. At follow-up, participants were classified as smokers if they indicated cigarette smoking on a weekly basis or more often. This definition for current smoking was consistent with data available from the CDAH and Bogalusa studies (Appendix 5).

#### 2.3.3.5 Carotid artery ultrasound studies

Ultrasound studies of the carotid arteries were introduced at the 2001 follow-up study and were performed using a Sequoia 512 ultrasound machine (Acuson, Mountain View, CA, USA) with 13.0 MHz linear array transducer. Studies were performed simultaneously between September 2001 and January 2002 at all five centres (Helsinki, Kuopio, Oulu, Tampere and Turku). The left common carotid artery was scanned by ultrasound technicians at each study centre following a standardised protocol. The image was focused on the posterior (far) wall and gain settings were used to optimise image quality. A resolution box function (zoom) was used to record an image of 25 mm in width and 15 mm height. A magnified image was recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. A moving scan with duration of five seconds which included the beginning of the carotid bifurcation and the common carotid artery was recorded and stored in digital format for subsequent off-line analysis.

The digitally stored scans were manually analysed by one reader blinded to the subjects' details. The measurements were performed using ultrasonic callipers. From the five second clip image, the best quality end-diastolic frame was selected (incident with the R-wave on a continuously recorded electrocardiogram). To measure carotid IMT, the image

was focused on the posterior (far) wall of the left carotid artery. At least four measurements of the far wall were taken approximately 10 mm proximal to the bifurcation to derive mean and maximum carotid IMT (Figure 15). All images were given a quality score (1 = excellent, 2 = average, 3 = poor) based on the presence of clear vessel boundaries. 97.4 % of images were rated excellent, 2.4 % were rated average, and 0.2% were rated as poor. To assess intra-individual reproducibility of ultrasound measurements, 57 subjects were re-examined three months after the initial visit (2.5 % random sample). The average absolute difference and standard deviation between measurements was 0.05±0.04 mm.

## 2.4. UNITED STATES DATA: THE BOGALUSA HEART STUDY

The Bogalusa Heart Study is a long-term community-based epidemiologic investigation of the early natural history of atherosclerosis and essential hypertension. Between 1973 and 2001, seven cross-sectional surveys of children and adolescents aged 4 to 17 years and six surveys of young adults aged 18 to 43 years (including some who had previously been examined in earlier studies) were conducted in a biracial (65% European-American, 35% African-American) population living in Ward 4 of Washington Parish, Louisiana.[324] The 1970 population of Ward 4 was 22,371, including approximately 5000 children and adolescents aged 5 to 17 years. Washington Parish includes the main semi-rural community of Bogalusa (Figure 18) situated 112 kilometres (70 miles) north of New Orleans. Bogalusa was chosen as the screening site as it has a stable, biracial paediatric population and is demographically comparable to many other small southern USA communities.[325]


**Figure 18.** Location of sampling area for the Bogalusa Heart Study: (A) location of Louisiana in the USA, and (B) location of Washington Parish and Bogalusa, LA.

### 2.4.1 SAMPLE SELECTION AND PARTICIPANTS

The eligible population for the initial cross-sectional study conducted during the 1973-1974 school year was all children aged 5 to 14 years attending any of the 14 schools (12 public, one parochial, one private) within Ward 4. In subsequent follow-ups conducted in 1976-1977, 1978-1979, 1981-1982, 1983-1984, 1987-1988, and 1992-1994, the age range was expanded to include all children aged 4 to 17 years attending any of the 14 schools within Ward 4.

Participation proportions in the cross-sectional surveys ranged from 80% to 92% for children and 60% to 65% for young adults. Due to the nature of the *panel design* of the Bogalusa study, consisting of repeated cross-sectional examinations every two to three years, serial observations from childhood and adolescence to adulthood were available for only some participants. The study cohort for this thesis was derived from individuals who participated in the 1984-1985 cross-sectional survey of 2666 children (baseline) and in the 2001-2002 cross-sectional survey of 1203 young adults (follow-up). This cohort was favoured as the baseline and follow-up measures were collected at approximately the same time-points as those in the CDAH and Young Finns studies. Of the children and adolescents that attended the 1984-1985 baseline survey, a subset of 379 participants (14% of those eligible from baseline) were also examined in the 2001-2002 follow-up as young adults. Figure 19 displays participation, response proportions, and the subset with measurements from the two time-points.

The study design and procedure were approved by the Louisiana State University and Tulane University Medical Center ethics and research committees. At baseline, written informed consent was obtained from parents of students; at follow-up, all participants provided written informed consent.

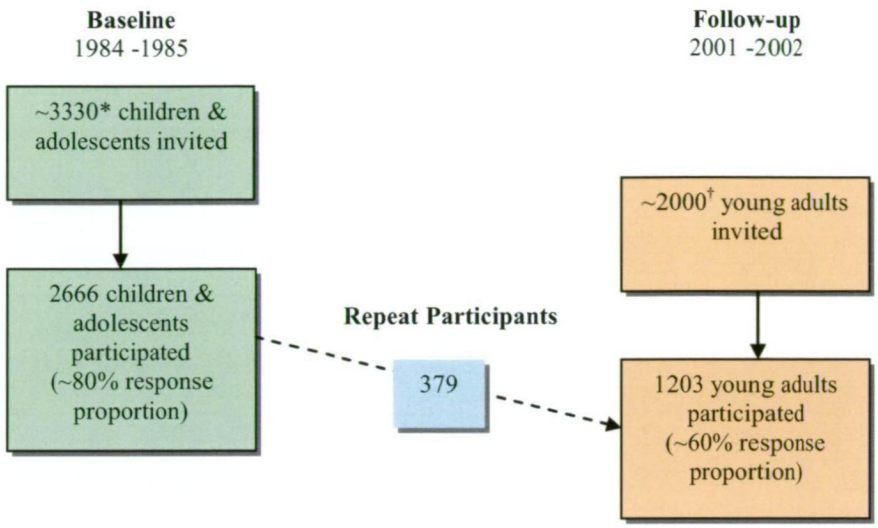


**Figure 19.** Participants, response proportions, and repeat participants in the 1984-1985 (baseline) and 2001-2002 (follow-up) cross-sectional surveys in the Bogalusa Heart Study. *This is an estimate of the total eligible population of children in Ward 4 at the time that was based on school lists. †The exact number is unknown. Invitations were sent to those known to still reside in the area; some participants not contacted directly were advised through word-of-mouth and participated.

## 2.4.2 DATA COLLECTION

*Baseline (1984-1985)*

Field staff received extensive training before data collection began. Performance criteria were established and assessed for all field staff on an on-going basis throughout data collection. Field personnel included two or three physicians, five registered nurses, six licensed practical nurses, one physician's assistant, six other staff members (project coordinator, nutritionist, clerical assistant), and several local volunteers.

A mobile research unit was converted from a three-bedroom house trailer (caravan) to aid examination of the children to be used in conjunction with facilities at each of the schools. In addition, an administrative office was established in Bogalusa with a laboratory area for preparing blood samples for shipment to the Louisiana State University Medical Centre laboratory in New Orleans for assaying.

Two-months before anticipated screening dates in each school, the project manager contacted the school's principal to obtain class lists. Five to six weeks before screening in each school, a letter of invitation (addressed to the parent) as well as a consent form and a health history form were given to home room teachers to hand out in class. Each child was asked to return the completed form the next day. Four weeks before screening, a follow-up of unreturned parental consents was conducted. Three weeks before screening, letters and consent forms were given to non-responders for a third time. One week before screening, parents of non-respondents were contacted by telephone or by home visits, and another consent form with stamped return envelopes was mailed or given to parents. Throughout the school year, the project manager visited each school to identify new students and obtain new consents for participation. Since a 12-14 hour fast was required for the lipid determinations, fasting instructions were sent to the parents the day before the child's screening session.

When possible, all examinations were conducted in school auditoria and in the mobile research unit. All children (approximately 30 to 40 per day) were examined between 8:30am and 12:00 noon. Figure 20 shows the flow of children throughout the clinical examination. The order of measurements followed a strict protocol. Blood samples were collected before participants were allowed brunch. Following brunch, measures of anthropometry (height, weight, upper-arm length, upper-arm circumference, and triceps and subscapular skin fold thicknesses), pubertal (maturation) stage (assessed by a physician), and blood pressure were collected.

*Follow-up (2001-2002)*

Field staff received extensive training before data collection began and were routinely assessed for adherence to protocols throughout data collection. Field personnel included one physician, one echocardiographer, two registered (or former registered) nurses, and four research assistants. Many of these staff had assisted in data collection of one or more previous examinations in the Bogalusa Heart Study. A permanent office was established in Bogalusa that was used for administrative and data collection purposes (this was formerly the administrative office in the baseline survey, which is equipped with a laboratory area for preparation of blood samples for shipment to the Tulane University School of Public Health and Tropical Medicine in New Orleans for assaying).

Approximately one-month before anticipated screening dates, participants were sent a letter of invitation to participate. Participants contact details are updated on an on-going basis, with any change of address or contact details indicated directly by the participant to the administrative office or study staff contacting next of kin to obtain the participant's new address or contact details.

All adults (approximately 6 to 7 per day) were examined between 7:30am and 12:00 noon. The flow of participants through the clinic essentially follows that from childhood. For example, blood and urine samples were collected first followed by brunch, and then anthropometric, blood pressure, echocardiography and ultrasound measurements, electro-cardiogram, and tonometry were obtained. Participants complete a questionnaire while at the clinic that enquires on demographic, health, family history, diet, and physical activity habits.

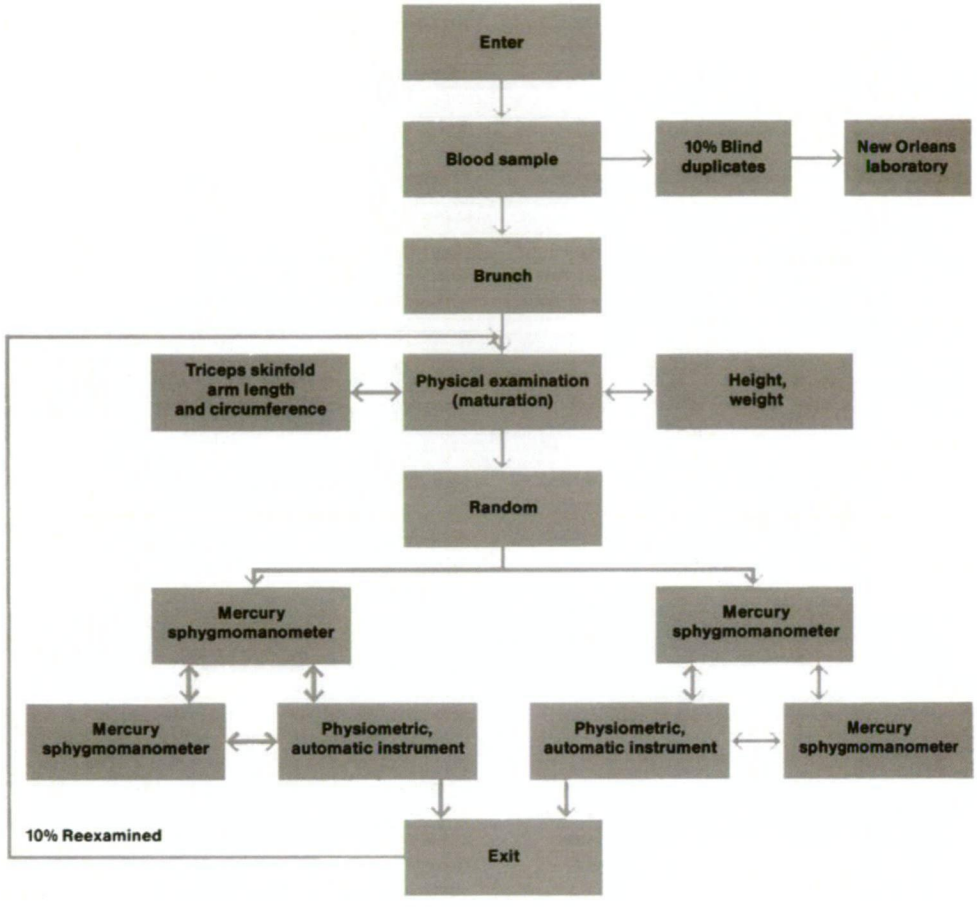


**Figure 20.** Flow of participants through the baseline clinical examination of the Bogalusa Heart Study. Adapted from Berenson et al.[33]

## 2.4.3 MEASURES

The baseline and follow-up measures relevant to the aims of this thesis are detailed below. Other measures and protocols from the cross-sectional and follow-up surveys of the Bogalusa study have been described in detail elsewhere.[209]

### 2.4.3.1 Blood collection and biochemistry

Standardised protocols were used by trained observers in all examinations.[209] Blood samples were centrifuged in Bogalusa and the samples sent in a cold pack box on the evening of each screening day by bus to the laboratory in New Orleans. In 1984 to 1985, cholesterol and triglyceride levels were measured with a Technicon Auto Analyzer II (Technicon Instrument

Corp, Tarrytown, NY), according to the laboratory manual of the LRC program.[313] In 2001 to 2002, cholesterol and triglycerides levels were determined by enzymatic procedures using an Hitachi 902 Automatic Analyzer (Roche Diagnostics, Indianapolis, IN). Lipoprotein cholesterol levels were analysed using a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis procedures.[326]

#### 2.4.3.2 Blood pressure

Blood pressure measurements at baseline and follow-up were obtained on the right arm, with participants in a relaxed, sitting position. Measurements of upper arm length and circumference were made to ensure selection of correct cuff size. Systolic and diastolic blood pressures were recorded at the first, fourth, and fifth Korotkoff phases using mercury sphygmomanometers. The phase five reading was used to denote diastolic pressure in the analyses. Three blood pressure readings were taken by each of two randomly assigned observers for a total of six measurements. The trained observers were blinded to each other's readings. The mean of the six replicate readings was used in the analyses.

#### 2.4.3.3 Anthropometry

At both baseline and follow-up, replicate height and weight measurements were collected. Height was measured to the nearest 0.1 cm using a manual height board. Weight was recorded to the nearest 0.1 kg using a balance beam metric scale (Detecto Scales, Inc, Webb City, MO). The average of the replicate measurements was used as the scores. Height and weight scores at baseline and follow-up were used to calculate BMI for each time-point.

#### 2.4.3.4 Smoking

Smoking behaviour at baseline and follow-up were ascertained from the Bogalusa 'Health Habits Questionnaire.'[327] Smoking behaviour was identified as: a child/adult who smokes at least one cigarette a week (current smoker); a child/adult who used to smoke at least one cigarette a week but no longer smokes cigarettes (former smoker); a child/adult who never experimented with cigarettes (never smoker); a child/adult who is currently experimenting with cigarettes (fewer than one cigarette per week, experimenter); a child/adult who at one time was experimenting (<1/week) with cigarettes but quit smoking (former experimenter). Participants were classified as smokers at baseline or follow-up if they currently smoked at

least one cigarette a week. This definition for current smoking was consistent with data available from the CDAH and Young Finns studies (Appendix 5).

#### 2.4.3.5 Carotid artery ultrasound studies

Trained sonographers performed B-mode ultrasound examinations using a Toshiba SonoLayer SSH 160A ultrasound system (Toshiba Medical, Tokyo, Japan) with a 7.5 MHz linear-array transducer, on participants in the supine position with the head slightly extended and turned to the opposite direction of the carotid artery being studied. Images of the common carotid (0 to 10 mm proximal to the origin of the carotid bifurcation, Figure 15), carotid bifurcation (covering the extent of the origin of the carotid bifurcation to the tip of the flow divider), and internal carotid (0 to 10 mm distal to the origin of the flow divider) segments from both the left and right carotid artery were recorded according to protocols previously described for the Atherosclerosis Risk in Communities (ARIC) Study.[154, 328, 329] Images were stored on VHS tapes and read by certified readers from the Division of Vascular Ultrasound Research using a semi-automated image processing program according to strict protocols.[329, 330] All readers were blinded to risk factor data. Maximum IMT measurements were taken from the far wall of both left and right common carotid, carotid bifurcation, and internal carotid segments. All measurements were recorded at 1 mm intervals along the segment of the imaged vessel at the time coinciding with the R wave of a continuously recorded electrocardiogram. Seventy-five participants underwent repeat ultrasound examinations 10 to 12 days after their initial visit to determine intra-individual reproducibility. The average absolute difference and standard deviation between measurements for all carotid IMT segments was 0.05±0.03 mm.

## 2.5. STATISTICAL ANALYSES

All statistical analyses were performed using STATA Versions 9.2 or 10 (STATA Corp, College Station, TX, USA), and statistical significance inferred at a 2-tailed P-value $\leq 0.05$. The statistical methods employed to address the aims of this study are covered in detail in subsequent chapters.

**KEY POINTS**

- This chapter described three population-based prospective cohort studies from Australia, Finland, and the United States - the primary data sources used in this thesis.
- The Childhood Determinants of Adult Health (CDAH) Study began in 1985 with baseline measures collected on a representative sample of 8498 Australian school children aged 7 to 15 years as part of the Australian Schools Health and Fitness Survey (ASHFS). Between 2004-2006, 2410 (28%) attended clinics held throughout Australia.
- The Cardiovascular Risk in Young Finns Study is an on-going epidemiologic study of atherosclerosis risk factors and precursors from childhood to adulthood. In 1980, 3596 children and adolescents aged 3, 6, 9, 12, 15 and 18 years participated in the first cross-sectional study. The 21-year follow-up was performed between September 2001 and January 2002, when 2283 participants from the original cohort (63%) participated in the study.
- The Bogalusa Heart Study is a biracial community-based investigation of the early natural history of CVD. The study cohort was derived from individuals who participated in the 1984-1985 cross-sectional survey of 2666 children and in the 2001-2002 cross-sectional survey of 1203 young adults. A total of 379 participants (14%) attended both surveys.
- Diverse biological and lifestyle measurements were collected at baseline and follow-up in each cohort. The key exposure measures central to this thesis include fasting blood lipid and lipoprotein levels collected at baseline.
- B-mode ultrasound was performed at follow-up in each cohort on the left common carotid artery to ascertain carotid IMT, a measure of preclinical atherosclerosis.

**Box 1.** Summary of key points from Chapter 2: Methods

# 3. FACTORS AFFECTING THE TRACKING OF BLOOD LIPID AND LIPOPROTEIN LEVELS FROM CHILDHOOD TO ADULTHOOD: THE CHILDHOOD DETERMINANTS OF ADULT HEALTH (CDAH) STUDY

## 3.1. INTRODUCTION

As outlined in section 1.5.6, the term *tracking* is used in epidemiological studies to describe the degree of consistency over time of an attribute, and is used to evaluate the longitudinal development of risk factors for chronic diseases.[246] From a paediatric perspective, tracking analyses are useful as they determine the ability to predict future values from measurements taken earlier in life, and to determine what risk factors, if any, should be the target of early treatment or prevention. Tracking is generally examined by the correlation between repeated measurements of the same attribute at two points in time, and the proportion of participants who remain within a specific group (dichotomies based on quantiles of the variable's distribution or clinically-defined cut-points) over time.

Because of their causal relationship with CVD in adulthood, blood lipid and lipoprotein levels have been investigated for tracking in the paediatric setting. In the past 25 years, 10 prospective studies have reported tracking of lipid and lipoprotein levels from childhood or adolescence into adulthood. These studies drew from five US cohorts, and a single cohort from each of Finland, Denmark, the Netherlands, Canada, and Australia. The data from these studies were provided in 20 publications.[160-162, 223, 224, 231-245] Appendix 7 provides an overview of the studies in terms of the sample size, observed population, length of follow-up, age at baseline and follow-up measurements, fasting status, statistical analyses, and summarises the main correlation findings from these studies. Appendix 8 provides a summary of studies that reported stability tracking by risk-group status. The studies showed considerable variation in terms of: sample size, ranging from 48 to 2446 participants; length of follow-up, which ranged from 3 to 27 years; age at baseline, with a range from 5 to 19 years; and the type of correlations and definition of risk-groups used in the statistical analyses. The Amsterdam Growth and Health Study[239-241] did not use fasting lipid samples in their analyses, and most studies measured only total cholesterol levels at both time-points. The Bogalusa Heart Study[162, 231, 233] and Cardiovascular Risk in Young Finns Study[161, 223]

reported tracking for LDL cholesterol, HDL cholesterol, and triglycerides in addition to total cholesterol.

While these studies found that youth levels correlate well with adult levels, they have shown that a substantial proportion of youth with high-risk levels will not have high-risk levels in adulthood and that a substantial proportion of those adults with high-risk levels had normal levels as children or adolescents.[245, 331] That is, there exists a reasonable amount of instability in the maintenance of abnormal blood lipid and lipoprotein levels from youth to adulthood. As such, these findings have called into question both the approach to, and utility of, screening for paediatric lipid disorders.[196, 220, 332] This has led to the study of factors that influence tracking in some of these studies.

Except for the period of infancy,[333] tracking of lipids and lipoproteins does not appear to be largely dependent on age at baseline measurement. However, the Bogalusa Heart study noted that tracking of HDL cholesterol improves after the age of eight years most likely due to the puberty-related decrease in HDL cholesterol.[162] Studies with multiple intervals between baseline and subsequent follow-up measurements show that tracking is time dependent.[161, 244] That is, the degree of tracking decreased as the time interval between measurements becomes longer. Using data from the studies detailed in Appendices 7 and 8, Figure 21 was constructed to show the weighted-mean Spearman's correlation coefficients from tracking studies[160-162, 223, 224, 232, 235-238, 243, 244] by length of follow-up for total cholesterol levels measured during childhood or adolescence and adulthood, and the weighted-mean percent remaining in the extreme fifth of the population distribution from tracking studies[161, 223, 224, 235, 237] for total cholesterol between childhood or adolescence and adulthood by length of follow-up. This graphical representation supports the conclusions of previous studies that suggest time between measurements has an effect on tracking of total cholesterol levels. Of the studies that reported tracking separately for males and females, males tend to track slightly better than females for total cholesterol, LDL cholesterol and triglycerides. The Bogalusa Heart study is the only study identified that examined the effect of race on tracking.[162, 231] While there was some modest evidence of better tracking of total cholesterol and LDL cholesterol levels in African-Americans compared with Caucasians, the authors noted that race did not appreciably affect the magnitude of the correlation coefficients or the proportions that were at risk at both time-points.[162]

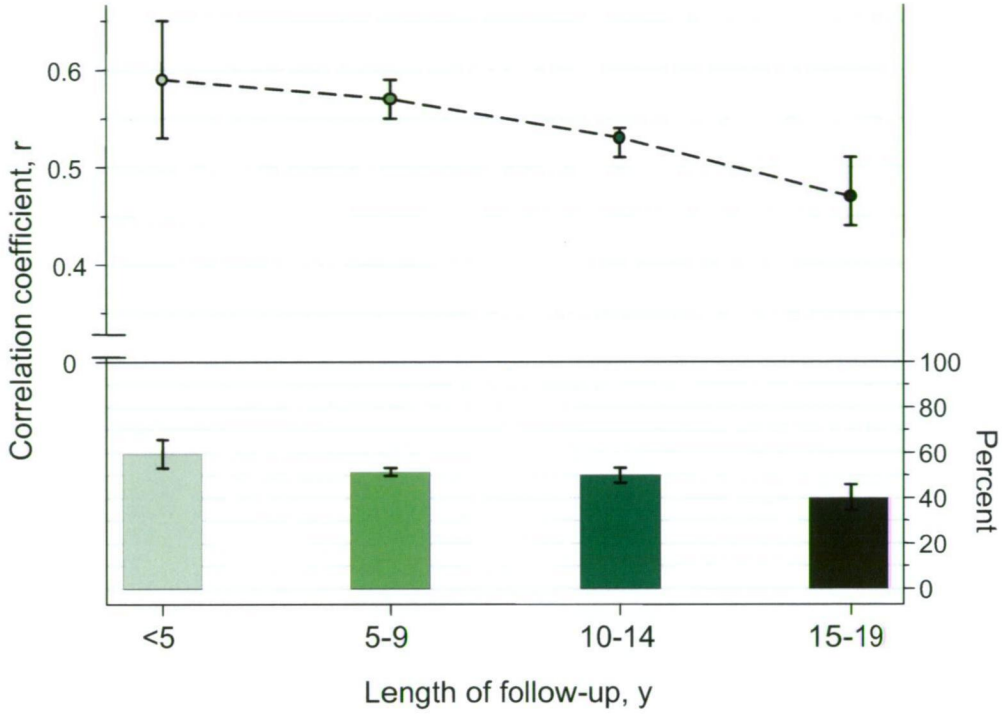


**Figure 21.** Weighted-mean Spearman's correlation coefficients from tracking studies[160-162, 223, 224, 232, 235-238, 243, 244] by length of follow-up for total cholesterol levels measured during childhood or adolescence and adulthood (upper figure portion); and weighted-mean percent remaining in the extreme fifth of the population distribution from tracking studies[161, 223, 224, 235, 237] for total cholesterol between childhood or adolescence and adulthood by length of follow-up (lower figure portion). Error bars represent 95% confidence intervals. Correlation coefficients and proportions from each study were weighted as N(study)/N(total) for each length of follow-up group. Sample sizes for each length of follow-up group were: <5 years (correlation N=240, percent N=238); 5-9 years (correlation N=3002, percent N=3000); 10-14 years (correlation N=5677, percent N=883); 15-19 years (correlation N=705, percent N=299). Only studies with fasting lipid measures were considered for this figure.

Two studies of those detailed in Appendices 7 and 8 examined the role of lifestyle-related factors in relation to tracking of lipid and lipoprotein levels between baseline and follow-up.[223, 224, 235] Their analyses compared participants with different tracking patterns. For example, participants who had high-risk levels at both baseline and follow-up were considered true positives; those who had high-risk levels at baseline but not at follow-up

were considered false-positives; those who acquired hypercholesterolemia as adults were considered as false-negatives; and those who did not have high-risk levels at both baseline and follow-up were considered as true negatives. For example, Stuhldreher et al.[224] and Orchard et al.[235] found in the Beaver County Lipid Study that those who had high-risk levels at baseline but not at follow-up had gained less weight, were less frequent smokers, and adhered to a diet low in saturated fat and cholesterol compared with those who acquired hypercholesterolemia in adulthood. In the six-year follow-up of the Young Finns Study, Porkka et al.[223] found that those who did not have high-risk levels at either time-point tended to gain less adiposity and were less likely to begin smoking compared with those who acquired a high-risk status. While those in the high-risk group at both time points gained more adiposity and were more likely to have begun smoking compared with those who were initially at high-risk. Collectively, these studies suggest some of the instability of blood lipid and lipoprotein tracking is the result of some participants adopting a healthier lifestyle, and others a less healthy lifestyle. Several studies have also reported that oral contraceptive use affects lipid levels,[334, 335] which may lead to poorer tracking in females who were not using oral contraceptives at the baseline examination.[160, 161, 336]

Lipid and lipoprotein levels are known to be subject to significant measurement variability.[308-310] Consistent with this knowledge, current and previous paediatric guidelines have recommended multiple measures before risk classification.[196, 220, 247] Most tracking studies used single lipid and lipoprotein measurements in their analyses and in doing so, may have underestimated the strength of relationship between child or adolescent levels and adult levels.[337] Those studies that had two measurements available during childhood or adolescence examined the effect of multiple measures on their tracking analyses. Use of two separate childhood measurements increased the amount of adult lipid variability explained in the Cardiovascular Risk in Young Finns Study by up to 50 percent,[161] while the prediction of adult dyslipidemia was markedly enhanced by multiple observations of LDL cholesterol in the Bogalusa Heart Study (Figure 22). These data suggest that studies that have used a single measurement at baseline may have substantially underestimated the strength of the relationship between childhood or adolescent levels and adult levels. This is underscored by data presented by Porkka et al.,[161] which show that regression toward the mean was the most important determinant of change in levels between childhood and adulthood, accounting for up to half of the variability. Although tracking would likely be improved by multiple child or adolescent measures, Lauer et al.[245] still noted that 25% to 45% of children with total

cholesterol levels greater than the 90th percentile on two successive occasions did not meet the criteria for intervention suggested by NCEP ATPIII[338] when they became adults. Although this study did not examine what impact the adoption of cholesterol-altering health behaviours might have had on misclassification, the findings further emphasise the point made earlier that while children with high lipid and lipoprotein levels have a greater risk of having elevated adult levels than their peers with lower levels, a substantial proportion of these children will not have adult levels that meet intervention criteria.

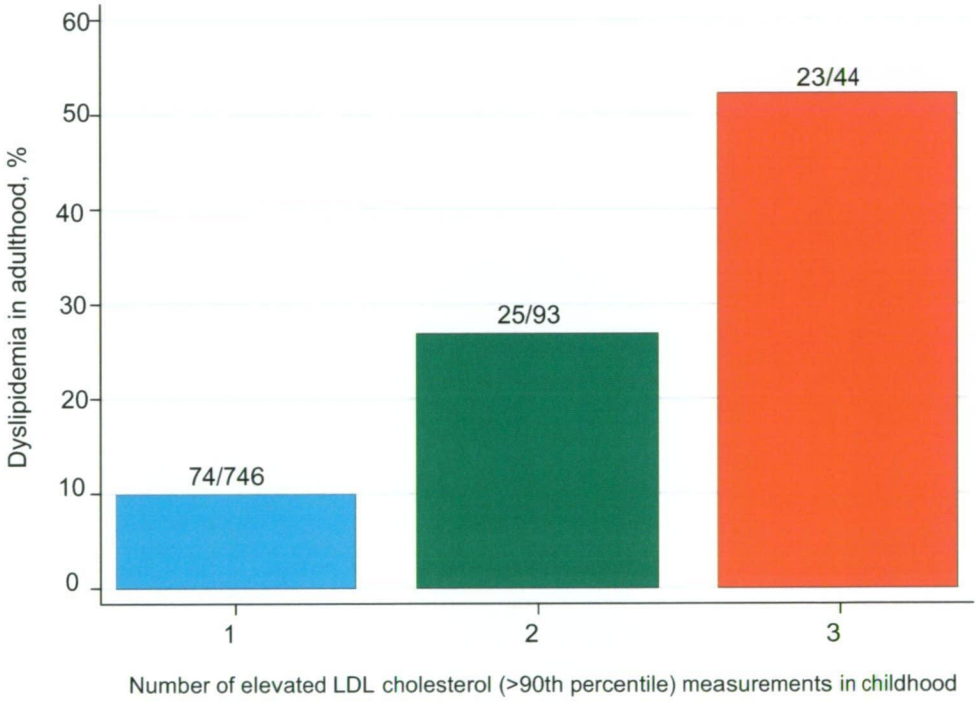


**Figure 22.** Prevalence of adulthood dyslipidemia by the number of elevated childhood LDL cholesterol measurements (adapted from Bao et al.).[231] Adult participants were classified as having dyslipidemia if their level of total cholesterol was ≥6.21 mmol/L, or LDL cholesterol ≥4.14 mmol/L, or HDL cholesterol <0.91 mmol/L, or triglyceride level was ≥4.52 mmol/L.

**KEY POINTS: What the literature tells us about the tracking of blood lipid and lipoprotein levels between childhood or adolescence and adulthood**

- Tracking is a term used to express the degree of consistency over time of an attribute, and is used to describe the ability to predict future values from early measurements.
- 10 prospective cohort studies have examined tracking of blood lipid and lipoprotein levels between childhood or adolescence and adulthood.
- These studies suggest moderate to strong tracking of total cholesterol, LDL cholesterol, and HDL cholesterol levels, and low to moderate tracking of triglyceride levels between childhood or adolescence and adulthood.
- Children with high lipid and lipoprotein levels have a greater risk of having elevated adult levels than their peers with lower levels, but a substantial proportion of these children will have adult levels that will not require intervention.
- Tracking appears to be only modestly influenced by age, sex, and race. However, tracking tends to diminish as length of time between baseline and follow-up measures increases.
- Changes in lifestyle factors (adiposity and smoking) that occur between youth and adulthood have an impact on whether an individual maintains, loses, or develops high-risk blood lipid and lipoprotein levels in adulthood.
- Single lipid or lipoprotein measurements increase the likelihood of misclassification and in turn, can decrease the strength of tracking.
- Key limitations of the tracking literature includes use of only one measurement to assign risk status at both baseline and follow-up in most studies, non-standardised definitions of the at-risk group, limited data available for LDL cholesterol, HDL cholesterol, and triglycerides, and the paucity of data examining lifestyle factors that may have contributed to individuals changing their risk status between childhood or adolescence and adulthood.

**Box 2.** Summary of literature that has examined tracking of blood lipid and lipoprotein levels from childhood or adolescence to adulthood.

## 3.2. AIMS

While the above literature demonstrates that tracking of total cholesterol levels from childhood or adolescence into adulthood has been examined in 10 studies, only two studies (the Bogalusa Heart Study[162, 231] and the Cardiovascular Risk in Young Finns Study[161, 223]) have examined tracking of fasting LDL cholesterol, HDL cholesterol and triglycerides levels from large population-based samples. In addition, only two studies[223, 224, 235] have examined some lifestyle factors of interest (adiposity, smoking, diet) that contribute to misclassification of adult lipid levels from child levels; one of these had data on total cholesterol levels only.[224, 235] Moreover, only two[234, 244] studies have examined tracking over a period of 20 years or more and only one of these studies has examined tracking of total cholesterol levels in an Australian population.[244] The aims were:

1. To study the tracking of total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels over a 20-year period from childhood to adulthood in the Australian Childhood Determinants of Adult Health (CDAH) study, and
2. To examine the effect of lifestyle changes on the tracking of these blood lipid and lipoprotein levels from childhood to adulthood.

## 3.3. METHODS

### 3.3.1 PARTICIPANTS

In this chapter, 539 participants with baseline (1985) and follow-up (2004-2006) blood samples from the CDAH study were examined. These participants were from the 9, 12, and 15 year old sub-sample that provided additional measures including bloods at the baseline survey (as detailed in section 2.3.1).

### 3.3.2 MEASURES

*Blood lipid and lipoprotein measurements*: Fasting blood lipids were measured at baseline and follow-up according to the protocols detailed in section 2.3.1.1.

*Adiposity*: Anthropometric measurements of height and weight at both time points were determined according to protocols outlined in section 2.3.1.3 and BMI calculated. Waist circumference was measured to the nearest 0.1 cm at both time-points but was taken at the level of the umbilicus during baseline examination and at the narrowest point between the lower costal border and iliac crest at follow-up. In 1985, skin fold thickness of the tricep, bicep, subscapular, and suprailiac was recorded to the nearest 0.1 mm on the right-side of the body using Holtain Callipers. Three readings were taken from each site, and the readings were then averaged. At follow-up, tricep, bicep, subscapular, and iliac crest skin folds were measured to the nearest 0.5 mm on the right-side of the body using Slim Guide callipers. Measurements were repeated a maximum of three times, or discontinued if the first two readings were unchanged. The average of the two closest readings was used as the location specific score. At follow-up, skin fold measures were truncated at 40mm, as this was the maximum limit of the callipers. For the truncated skin fold measures, skin fold values were imputed from BMI and waist circumference using Tobit Regression[339, 340]

*Smoking status:* As described in section 2.3.1.4, participants were classified as smokers at baseline and follow-up if they indicated cigarette smoking on a weekly basis or more often. This was the most consistent indicator of smoking available at both time-points.

*Cardiorespiratory fitness:* Cardiorespiratory fitness was estimated at baseline and follow-up as physical working capacity at a heart rate of 170 beats per minute on a friction-braked

bicycle ergometer (Monark Exercise AB, Sweden) according to standard protocols.[341] The test protocol comprised three successive sub-maximal workloads of three minutes at baseline, and four minutes at follow-up. The workloads were selected by the technicians on an individual basis to induce steady-state heart rate responses from the participant during the first, second and third workloads within the ranges of 115-130, 130-145, and 145-160 beats per minute respectively. Physical work capacity at 170 beats per minute was estimated by extrapolating the line of best fit from the heart rates recorded at each sub-maximal workload.[342] The measure of baseline and follow-up cardiorespiratory fitness used in this study was expressed in relative terms as watts per kg (W/kg) of lean body mass. Relative cardiorespiratory fitness is preferred because the absolute workload (watts) achieved is a function of muscle mass – best estimated by inclusion of lean body mass.[343] Body density was estimated from the log of the sum of four skin folds using the regression equations of Durnin and Rahaman for 12 and 15 year old children,[344] and Brook for 9 year old children[345] at baseline, and Durnin and Womersley[346] for adults at follow-up. Calculations of body fat were made using the equation specified by Siri.[347] Lean body mass was then estimated by subtracting fat mass from total body mass.

*Socioeconomic position:* Data on parental education (low = school only, medium = trade/vocational, and high = university) collected retrospectively at follow-up was used as an indicator of individual-level socioeconomic position (SEP) at baseline.[348] At follow-up self-reported highest level of education (low = school only, medium = trade/vocational, and high = university) was used as the indicator of SEP.

*Dietary data*: Participants aged 12 and 15 years at baseline recorded their food consumption over a 24-hour period.[349] The students, in groups of four or five, were given a record diary by a data collector and shown how to record their food intake with practical examples of food measurement. As a practice exercise, they recorded the breakfast they had eaten that morning. This recording was then checked for the detail necessary to allow coding and conversion into nutrient intake and the participants given feedback. The 24-hour recording period began at the end of the briefing session. Once the 24-hour recoding period had elapsed, each student was interviewed individually and their record diary checked to clarify missing or illegible information. A number of dietary variables were calculated from this record that included the proportion of dietary energy from saturated fat.

At follow-up, food habits and food frequency questionnaires were completed.[350] Questions on fat intake included: the type of milk usually consumed (1 = skimmed milk, 2 = low/reduced fat milk, 3 = whole milk); the frequency of trimming fat from meat (1 = I don't eat meat, 2 = usually, 3 = sometimes, 4 = never/rarely); and the type of spread usually used on bread (1 = I do not use any spread, 2 = spread other than butter or margarine, 3 = margarine, 4 = butter). The food frequency questionnaire does not allow the proportion of dietary energy from saturated fat to be derived (that was available from the baseline survey), however, these variables have been shown to provide reliable and valid estimates[351] that are positively correlated with higher saturated fat intake.[352, 353] Scores from these three questions linked to fat intake were summed to derive a single variable of dietary behaviour relating to fat intake at follow-up.[340]

*Use of hormonal contraceptives in females*: Use of hormonal contraception (HC) was not ascertained in adolescent females at baseline. At follow-up, females were asked if they were currently using any of the following hormonal contraceptives: combined oral contraceptives, minipill, weekly contraceptive patch, progestagen, progestagen injection, progestin releasing intrauterine device, progestin releasing implant, or other.

### 3.3.3 STATISTICAL ANALYSES

*Loss to follow-up*

Comparisons between baseline characteristics of participants and non-participants at follow-up were performed using logistic regression. Participation (yes/no) was used as the binary dependent variable in these analyses. Both age and sex were included in models to account for differences in these variables between participants and non-participants.

*Tracking of blood lipids from childhood to adulthood*

Tracking was estimated in two ways: (1) Spearman's rank-order correlation coefficients; and (2) the proportion of participants who remained in high-risk categories in childhood and adulthood. Two approached were used to classify child and adolescent levels. First, paediatric NCEP or NHANES cut-points (Table 7)[196, 251] that were shown to be the best predictors of adult dyslipidemia in data from three cohorts[331] (see results detailed in chapter 4), and the adult cut-points stipulated in the third report of the National Cholesterol Education Program's Adult Treatment Panel (NCEP ATPIII).[304] Second, the upper fifth of the population

distribution in childhood and adulthood (lower fifth in the case of HDL cholesterol). The first approach provides clinically relevant information, while the second approach is most often used in the existing literature, which allows for comparison.

**Table 7.** Paediatric[196, 251] and adult[304] blood lipid and lipoprotein cut-points (mmol/L) used in the analyses

| | Paediatric | | | | | Adult |
|---|---|---|---|---|---|---|
| | NCEP[196] | NHANES[251] | | | | NCEP[304] |
| | | 12y | | 15y | | |
| | | M | F | M | F | |
| Total cholesterol | | | | | | |
| Normal | <4.40 | - | - | - | - | <5.18 |
| Borderline high | 4.40-5.17 | - | - | - | - | 5.18-6.21 |
| High | ≥5.18 | - | - | - | - | ≥6.22 |
| LDL cholesterol | | | | | | |
| Normal | <2.85 | - | - | - | - | <3.37 |
| Borderline high | 2.85-3.36 | - | - | - | - | 3.37-4.13 |
| High | ≥3.37 | - | - | - | - | ≥4.14 |
| HDL cholesterol* | | | | | | |
| Normal | >1.56 | ≥1.70 | ≥1.48 | ≥1.55 | ≥1.49 | ≥1.55 |
| Borderline low | 1.56-0.91 | 1.69-1.14 | 1.47-1.04 | 1.54-1.05 | 1.48-1.04 | 1.54-1.036 |
| Low | <0.91 | ≤1.13 | ≤1.03 | ≤1.04 | ≤1.03 | <1.036 |
| Triglycerides | | | | | | |
| Normal | <1.02 | - | - | - | - | <1.70 |
| Borderline high | 1.02-1.46 | - | - | - | - | 1.70-2.25 |
| High | ≥1.47 | - | - | - | - | ≥2.26 |

Abbreviations: y, age in years; M, males; F, females

*As per recommendations from Magnussen et al.,[331] (see chapter 4) NHANES cut-points[251] for HDL cholesterol were used for those aged 12 or 15 years at baseline.

NCEP cut-points were used for those aged 9 years because NHANES cut-points were not available for those aged <12 years.

To convert total cholesterol, LDL cholesterol and HDL cholesterol to mg/dl, multiply values by 38.67; to convert triglyceride values to mg/dl, multiply values by 88.5.

*Factors affecting tracking of blood lipid levels from childhood to adulthood*

In order to determine the factors that might impact on the tracking of childhood lipid and lipoprotein levels, participants were divided into four tracking groups depending on their status (using the clinical cut-points displayed in Table 7) in childhood and adulthood. Participants who remained in high-risk categories at both time-points were considered as *true positives*; those who were high-risk in childhood but not at follow-up were considered as *false positives*; those who were not high-risk in childhood but were in adulthood were considered as *false negatives*; and those that did not have high-risk levels at both time-points were considered *true negatives* (Figure 23). This is the approach that has been adopted in other studies that have examined factors that influence tracking (and thus prediction) of lipid and other risk factor levels between two time-points.[223-225, 235] In separate analyses for each lipid or lipoprotein, logistic regression was applied to examine the effect of changes in lifestyle-related variables (adiposity measures, cardiorespiratory fitness, social mobility, smoking, and saturated fat intake) between childhood and adulthood that increased the likelihood of being false positives (unstable tracking) as opposed to true positives (stable tracking) amongst those who were high-risk in childhood, and to identify the factors that increased the odds of being false negatives (unstable tracking) as opposed to true negatives (stable tracking) amongst those who were low-risk in childhood. The logistic regression models were adjusted for age and sex. If multiple lifestyle variables were found to be associated with tracking of a single lipid or lipoprotein variable, a model that included all significant lifestyle variables in addition to age and sex were fit to examine for independent effects. To examine if sex modified the relationships between lifestyle change and tracking, sex by lifestyle change interaction terms were fitted to each model. There were no significant interactions.

Changes in continuous lifestyle-related variables (adiposity measures, cardiorespiratory fitness, and saturated fat intake) were analysed using the difference (adult minus child) of age- and sex-specific z-scores at each time-point. This approach was favoured as it accounts for any differences in measurement protocols between surveys and allows the full spectrum of change to be examined (small and large). For change in SEP, a social mobility variable was created,[348] using highest level of parental education at baseline and highest level of own education at follow-up to derive change or stability in SEP as follows: persistently low (low at baseline and follow-up), persistently medium (medium at baseline and follow-up), persistently high (high at baseline and follow-up), upwardly mobile

(moving from low at baseline to medium or high at follow-up, or medium at baseline to high at follow-up), and downwardly mobile (moving from high at baseline to medium or low at follow-up, or from medium at baseline to low at follow-up).

Because the baseline level of the lifestyle risk factor may have an effect on the magnitude of change, models that included the baseline variable as a covariate, that examined for interactions between the baseline variable and change were also fitted. There were no significant interactions. Examining the data either with or without the baseline variable in the model produced essentially similar results and did not change the conclusions. Because of this, the results are presented for the more parsimonious model (without the baseline variable as a covariate) because power was a consideration in some of the analyses.

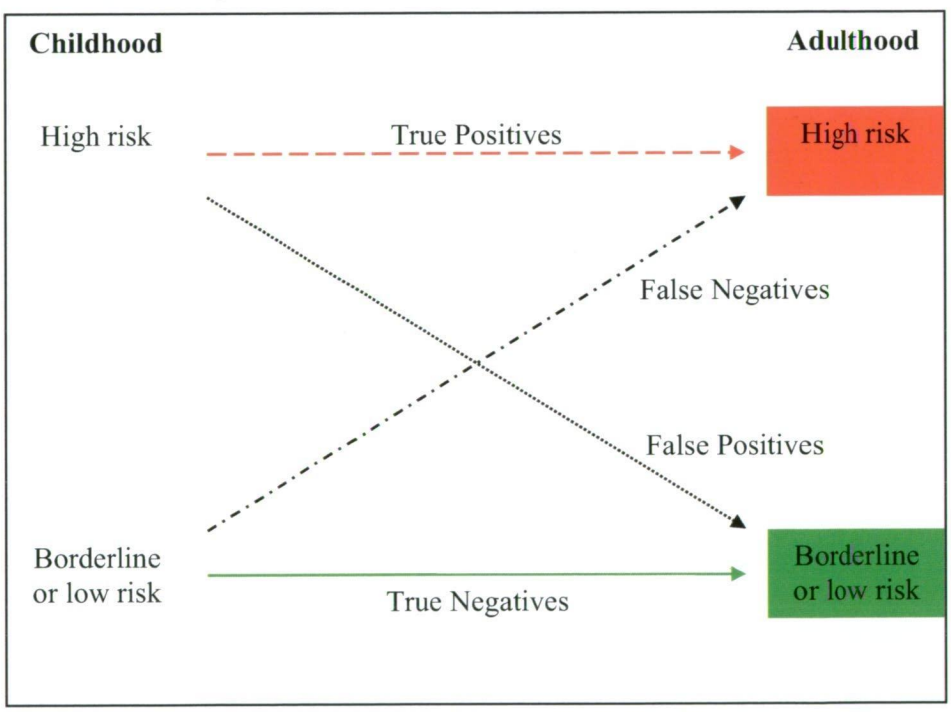


**Figure 23.** Schematic representation of blood lipid and lipoprotein categorical tracking possibilities from childhood to adulthood in the CDAH study.

## 3.4. RESULTS

*Comparison of baseline characteristics of participants and non-participants*

Participants (30.5% of those eligible from baseline) were older (mean 11.9 vs. 11.7 years, P=0.03), were more likely to be female (50.1 vs. 45.4%, P=0.07), had lower adiposity (mean BMI, 18.5 vs. 18.7 kg/m$^2$, P<0.01; waist, 65.2 vs. 65.5 cm, P=0.02; sum of skin folds, 36.0 vs. 37.6 mm, P<0.01), were less likely to be smokers (10.8 vs. 14.2%, P<0.01), and were more likely to be of high SEP in childhood (28.2 vs. 21.0%, P<0.01) compared with non-participants. Participants were however similar to non-participants with respect to childhood lipid and lipoprotein levels, cardiorespiratory fitness, and saturated fat intake as a percentage of total daily intake.

*Participant characteristics*

Key child and adult characteristics of male and female participants are summarised in Table 8. With the exception of HDL cholesterol, females tended to have a more adverse lipid and lipoprotein profile in childhood than males, but this trend was reversed in adulthood. Males had higher fitness levels and lower sum of skin folds at baseline than females. At follow-up, males had higher BMI and waist circumference but lower sum of skin fold thickness compared with females. A higher proportion of adult males were smokers, used whole fat milk, and more seldom trimmed fat from meat compared with adult females. Mean length of follow-up between the two surveys was 20.0±0.5 years.

**Table 8.** Characteristics of 539 CDAH participants* who had blood lipid and lipoprotein measurements at baseline and follow-up

| Characteristic | N | Males | N | Females |
|---|---|---|---|---|
| Childhood | | | | |
| Age, y | 269 | 11.9±2.5 | 270 | 11.9±2.5 |
| Total cholesterol, mmol/L | 269 | 4.41±0.73 | 270 | 4.62±0.78 |
| LDL cholesterol, mmol/L | 266 | 2.65±0.65 | 267 | 2.81±0.75 |
| HDL cholesterol, mmol/L | 267 | 1.43±0.29 | 267 | 1.48±0.29 |
| Triglycerides, mmol/L | 269 | 0.65(0.50,0.84) | 270 | 0.66(0.54,0.85) |
| BMI, $kg/m^2$ | 269 | 18.4±2.8 | 270 | 18.6±2.9 |
| Waist circumference, cm | 269 | 66.7±8.6 | 270 | 63.7±7.8 |
| Skin folds (sum of 4), mm | 266 | 31.0±15.3 | 269 | 41.0±17.6 |
| Fitness, W/kg | 255 | 3.09±0.60 | 250 | 2.50±0.57 |
| Energy intake, kJ† | 165 | 10429±3451 | 169 | 7742±2294 |
| % kJ fat† | 165 | 36.7±6.4 | 169 | 36.9±6.8 |
| % kJ saturated fat† | 165 | 16.1±3.8 | 169 | 15.8±3.7 |
| Current smokers, % | 263 | 10.7 | 263 | 11.0 |
| Hormonal contraceptive users, % | - | - | NA | NA |
| Parental education | | | | |
| School only | 102 | 41.8 | 94 | 36.7 |
| Trade/vocational certificate | 74 | 30.3 | 73 | 28.5 |
| University | 68 | 27.9 | 89 | 34.8 |
| Adulthood | | | | |
| Age, y | 269 | 32.4±2.6 | 270 | 32.4±2.5 |
| Total cholesterol, mmol/L | 269 | 5.03±0.96 | 270 | 4.83±1.04 |
| LDL cholesterol, mmol/L | 264 | 3.16±0.81 | 270 | 2.86±0.92 |
| HDL cholesterol, mmol/L | 269 | 1.29±0.28 | 270 | 1.54±0.33 |
| Triglycerides, mmol/L | 269 | 1.10(0.70,1.60) | 270 | 0.80(0.60,1.10) |
| BMI, $kg/m^2$ | 254 | 26.7±3.9 | 235 | 25.0±5.6 |
| Waist circumference, cm | 254 | 90.5±9.7 | 236 | 78.5±11.6 |
| Skin folds (sum of 4), mm | 254 | 66.0±24.7 | 236 | 78.7±32.1 |
| Fitness, W/kg | 232 | 3.07±0.61 | 197 | 3.02±0.70 |
| Whole milk, % | 250 | 47.6 | 246 | 31.3 |
| Usually use butter on bread, % | 251 | 22.7 | 250 | 24.4 |
| Never or rarely trim fat from meat, | 250 | 16.4 | 251 | 7.2 |

| Characteristic | N | Males | N | Females |
|---|---|---|---|---|
| % | | | | |
| Current smokers, % | 254 | 20.1 | 253 | 14.2 |
| Hormonal contraceptive users, % | - | - | 270 | 33.7 |
| Education | | | | |
| School only | 100 | 40.2 | 129 | 48.9 |
| Trade/vocational certificate | 83 | 33.3 | 53 | 20.1 |
| University | 66 | 26.5 | 82 | 31.1 |

*Changing Ns are the result of missing data for some participants.

† Baseline dietary variables not measured for those aged 9 years.

Statistics are means ± SD or median (interquartile range) for continuous variables or percent for dichotomous variables.

To convert total cholesterol, LDL cholesterol and HDL cholesterol to mg/dl, multiply values by 38.67; to convert triglyceride values to mg/dl, multiply values by 88.5.

*Tracking of blood lipids and lipoproteins from childhood to adulthood*

*Correlation coefficients*

Spearman's correlation coefficients for tracking of blood lipid and lipoproteins from childhood to adulthood are presented in Table 9. Overall, rank-order tracking was strongest for LDL cholesterol in both males and females, followed by total cholesterol and HDL cholesterol with triglycerides displaying the lowest rank-order tracking. Tracking of blood lipid and lipoprotein levels was generally consistent for males and females with the exception of triglycerides, where baseline levels in males tended to track more strongly into adulthood than baseline levels in females. No clear age differences were observed in correlation coefficients, although for triglycerides, the lowest values were seen in males and females aged 9 years at baseline.

**Table 9.** Spearman rank correlation coefficients for tracking of blood lipid and lipoprotein measures from childhood (1985) to adulthood (2004-2006) (N in parentheses)

| | Age (y) in 1985 | | | |
|---|---|---|---|---|
| Variable | 9 | 12 | 15 | All |
| Total cholesterol | | | | |
| Males | 0.58† (95) | 0.58† (86) | 0.45† (88) | 0.54† (269) |
| Females | 0.55† (95) | 0.49† (85) | 0.56† (90) | 0.54† (270) |
| LDL cholesterol | | | | |
| Males | 0.59† (92) | 0.65† (85) | 0.50† (85) | 0.58† (262) |
| Females | 0.63† (94) | 0.56† (83) | 0.60† (90) | 0.60† (267) |
| HDL cholesterol | | | | |
| Males | 0.50† (94) | 0.55† (86) | 0.36† (87) | 0.47† (267) |
| Females | 0.56† (95) | 0.26* (84) | 0.51† (90) | 0.47† (269) |
| Triglycerides | | | | |
| Males | 0.33† (95) | 0.44† (86) | 0.48† (88) | 0.41† (269) |
| Females | 0.16 (95) | 0.38† (85) | 0.28† (90) | 0.26† (270) |

*P<0.05, †P<0.01.

*Stability*

Figure 24 shows the proportions of males and females who had high-risk (using clinically-defined cut-points) blood lipid and lipoprotein levels at both time-points. Tracking within the high-risk categories was generally low for all lipids and lipoproteins with the exception of HDL cholesterol in males. Males showed greater stability in high-risk categories compared with females. Among males and females classified as having high triglyceride levels in childhood, most (79% and 98% respectively) had normal levels in adulthood. For comparison, Table 10 shows the distribution of childhood risk categories by adult risk categories. Approximately 40-70% of participants who had high-risk total and LDL cholesterol levels as adults also had high risk levels in childhood. The majority of males and females with high-risk adult HDL cholesterol or triglyceride levels had normal levels as children.

In addition to tracking by paediatric risk categories, Table 11 shows the probability of remaining in the extreme fifth of each blood lipid distribution from childhood to adulthood. Of the participants in the extreme fifth of the distribution at childhood, 40 to 50% were also in the extreme fifth in adulthood for most blood lipids and lipoproteins. The one exception being HDL cholesterol for females, with only 25% having extreme (low) levels at both time-points.

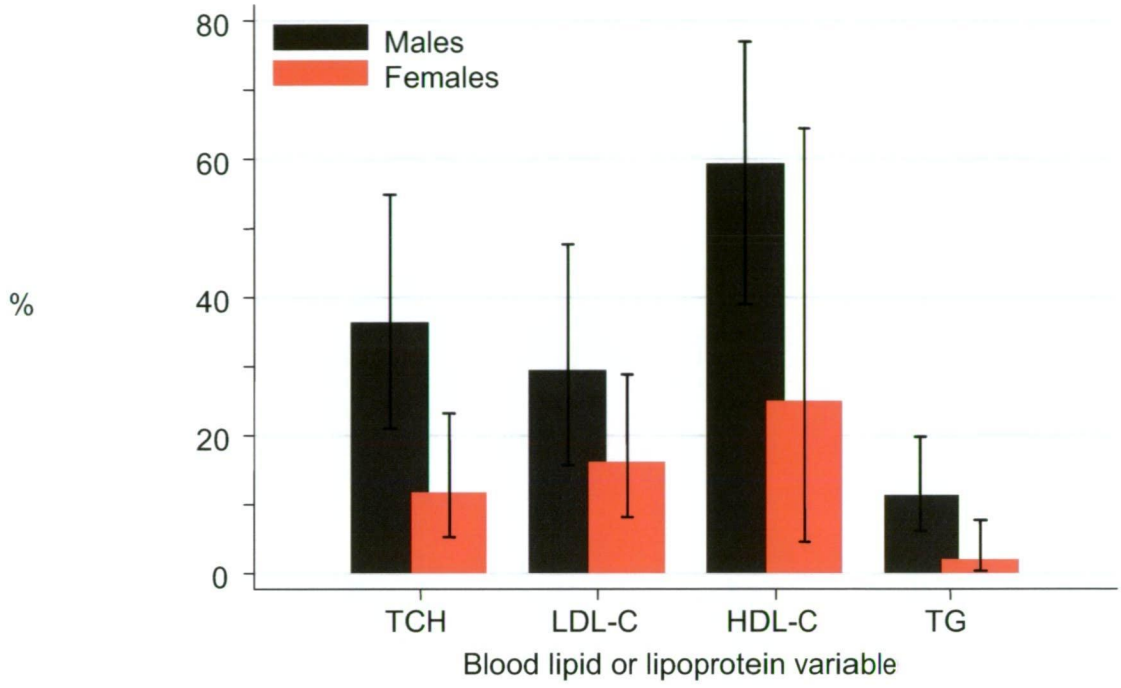


**Figure 24.** Proportions of males and females who had high-risk levels in childhood and adulthood for total cholesterol (TCH), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Error bars represent 95% confidence intervals. High-risk defined according to paediatric NCEP or NHANES cut-points,[196, 251] and adult NCEP ATPIII cut-points as detailed in Table 7.[304]

**Table 10**. Tracking of blood lipids from childhood to adulthood expressed as proportions in pediatric[196, 251] levels by adult levels[304].

| | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Child classification | | | | | | Child classification | | | | | |
| | Normal | | Borderline-high (low) | | High (low) | | Normal | | Borderline-high (low) | | High (low) | |
| Adult classification | N | % | N | % | N | % | N | % | N | % | N | % |
| Total cholesterol | | | | | | | | | | | | |
| Normal | 106 | 68.0 | 41 | 26.3 | 9 | 5.8 | 99 | 54.1 | 60 | 32.8 | 24 | 13.1 |
| Borderline-high | 28 | 33.3 | 44 | 52.4 | 12 | 14.3 | 12 | 16.7 | 31 | 43.1 | 29 | 40.3 |
| High | 5 | 17.2 | 12 | 41.4 | 12 | 41.4 | 1 | 6.7 | 7 | 46.7 | 7 | 46.7 |
| LDL cholesterol | | | | | | | | | | | | |
| Normal | 131 | 78.9 | 26 | 15.7 | 9 | 5.4 | 134 | 67.0 | 41 | 20.5 | 25 | 12.5 |
| Borderline-high | 32 | 45.1 | 24 | 33.8 | 15 | 21.1 | 12 | 22.2 | 20 | 37.0 | 22 | 40.7 |
| High | 8 | 32.0 | 7 | 28.0 | 10 | 40.0 | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| HDL cholesterol | | | | | | | | | | | | |
| Normal | 17 | 41.5 | 24 | 58.5 | 0 | 0.0 | 67 | 53.2 | 59 | 46.8 | 0 | 0.0 |
| Borderline-low | 39 | 23.1 | 119 | 70.4 | 11 | 6.5 | 30 | 23.1 | 94 | 72.3 | 6 | 4.6 |
| Low | 3 | 5.3 | 38 | 66.7 | 16 | 28.1 | 2 | 15.4 | 9 | 69.2 | 2 | 15.4 |
| Triglycerides | | | | | | | | | | | | |
| Normal | 126 | 58.9 | 11 | 5.1 | 77 | 36.0 | 133 | 53.6 | 17 | 6.9 | 98 | 39.5 |
| Borderline-high | 9 | 36.0 | 7 | 28.0 | 9 | 36.0 | 9 | 69.2 | 3 | 23.1 | 1 | 7.7 |
| High | 18 | 60.0 | 1 | 3.3 | 11 | 36.7 | 7 | 77.8 | 0 | 0.0 | 2 | 22.2 |

Row totals for percentages may not add to 100 because of rounding.

**Table 11.** Proportions (%) remaining in the extreme fifth* of each blood lipid distribution from childhood to adulthood

| | Males | Females | All |
|---|---|---|---|
| Total cholesterol | 46.3 | 38.9 | 42.6 |
| LDL cholesterol | 40.4 | 50.0 | 45.2 |
| HDL cholesterol | 52.0 | 25.0 | 38.2 |
| Triglycerides | 44.6 | 42.6 | 43.6 |

*Highest fifth for total cholesterol, LDL cholesterol, and triglycerides; lowest fifth for HDL cholesterol.

*Factors affecting tracking of blood lipid and lipoprotein levels from childhood to adulthood*

The effects of changes in lifestyle-related variables on tracking patterns of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides are displayed in Table 12. For LDL cholesterol, HDL cholesterol and triglycerides, participants who acquired high-risk levels in adulthood (false negatives) had significantly increased BMI, waist circumference, and sum of skin fold thickness between surveys relative to their peers compared with those who maintained normal-risk levels (true negatives).

Of the remaining risk factors examined, false negative HDL cholesterol participants significantly decreased cardiorespiratory fitness between survey intervals compared with true negatives. Higher proportions of true positives were persistent smokers or had begun smoking compared with false positives for total cholesterol and HDL cholesterol respectively. False negative participants for HDL cholesterol were less likely to have moved upward in SEP status from childhood to adulthood compared with true negatives. Table 12 shows that females who did not have adverse total cholesterol and triglyceride levels at baseline but did at follow-up (false negatives) were more likely to be current HC users than those who did not have adverse levels at both time-points (true negatives). In addition, females with adverse total cholesterol levels at both time-points (true positives) were more likely to be current HC users than those who had adverse levels in childhood but not in adulthood (false positives).

In multivariable models that compared total cholesterol in participants classified as true positive or false positive, both change in waist circumference ($P=0.05$) and persistent smoking ($P<0.01$) remained significant (model $R^2=7.7\%$). For HDL cholesterol, multivariable models comparing true positives with false positives showed that the differences in change in waist circumference ($P<0.01$) and the proportion of participants that had begun smoking ($P<0.01$) remained statistically significant (model $R^2=10.8\%$). In multivariable models for HDL cholesterol that compared false negatives and true negatives, change in waist circumference ($P<0.01$) remained significant whereas the effects for change in cardiorespiratory fitness ($P=0.10$) and upward social mobility ($P=0.07$) were attenuated marginally (model $R^2=13.9\%$).

**Table 12**. Factors affecting the tracking of blood lipid and lipoprotein levels from childhood to adulthood

| Blood lipid or lipoprotein variable<br>Lifestyle variable | True Positives<br>N | <br>mean (SD) or % | False positives<br>N | <br>mean (SD) or % | False negatives<br>N | <br>mean (SD) or % | True negatives<br>N | <br>mean (SD) or % |
|---|---|---|---|---|---|---|---|---|
| Total cholesterol | | | | | | | | |
| Δ BMI | 19 | 0.26 (0.85) | 158 | 0.07 (0.91) | 55 | 0.31 (0.73) | 257 | 0.11 (0.85) |
| Δ waist circumference | 19 | 0.43 (0.97) | 159 | 0.01 (0.85)* | 55 | 0.18 (0.77) | 257 | 0.07 (0.93) |
| Δ skin folds (sum of 4) | 19 | 0.23 (0.86) | 159 | -0.03 (1.03) | 54 | 0.37 (0.86) | 254 | 0.13 (1.00) |
| Δ cardiorespiratory fitness | 12 | 0.07 (1.47) | 129 | 0.11 (1.14) | 40 | 0.03 (1.12) | 219 | -0.05 (1.23) |
| Δ saturated fat intake | 9 | -0.03 (1.52) | 84 | -0.06 (1.30) | 48 | 0.19 (1.50) | 166 | -0.03 (1.42) |
| Smoking status | | | | | | | | |
| Never smoker | 10 | 53 | 127 | 80 | 39 | 68 | 204 | 79 |
| Stopped smoking | 1 | 5 | 7 | 4 | 5 | 9 | 19 | 7 |
| Began smoking | 5 | 26 | 22 | 14 | 9 | 16 | 28 | 11 |
| Smoker at both time-points | 3 | 16 | 3 | 2* | 4 | 7 | 8 | 3 |
| Social mobility | | | | | | | | |
| Persistently low | 3 | 17 | 23 | 15 | 7 | 12 | 33 | 13 |
| Persistently moderate | 3 | 17 | 18 | 12 | 7 | 13 | 24 | 10 |
| Persistently high | 0 | 0 | 4 | 3 | 1 | 2 | 15 | 6 |
| Downwardly mobile | 7 | 39 | 57 | 37 | 24 | 43 | 88 | 36 |
| Upwardly mobile | 5 | 28 | 51 | 33 | 17 | 30 | 88 | 36 |
| Female participants currently using HC | 12 | 57 | 10 | 30* | 13 | 52 | 56 | 29† |
| LDL cholesterol | | | | | | | | |
| Δ BMI | 18 | 0.39 (0.67) | 127 | 0.02 (0.88) | 56 | 0.47 (0.81) | 279 | 0.08 (0.84)† |
| Δ waist circumference | 18 | 0.55 (0.83) | 127 | -0.03 (0.84)* | 56 | 0.38 (0.92) | 280 | 0.05 (0.89)† |
| Δ skin folds (sum of 4) | 18 | 0.34 (0.74) | 127 | -0.03 (0.97) | 55 | 0.49 (0.91) | 277 | 0.11 (0.98)† |
| Δ cardiorespiratory fitness | 10 | 0.01 (1.12) | 101 | 0.21 (1.06) | 41 | -0.10 (1.24) | 241 | -0.03 (1.25) |
| Δ saturated fat intake§ | 7 | 0.03 (0.88) | 65 | 0.00 (1.35) | 45 | 0.20 (1.5) | 185 | -0.04 (1.4) |

| Blood lipid or lipoprotein variable<br>Lifestyle variable | True Positives<br>N | <br>mean (SD) or % | False positives<br>N | <br>mean (SD) or % | False negatives<br>N | <br>mean (SD) or % | True negatives<br>N | <br>mean (SD) or % |
|---|---|---|---|---|---|---|---|---|
| Smoking status | | | | | | | | |
| Never smoker | 10 | 59 | 99 | 77 | 42 | 72 | 222 | 79 |
| Stopped smoking | 2 | 12 | 6 | 5 | 4 | 7 | 20 | 7 |
| Began smoking | 4 | 24 | 20 | 16 | 7 | 12 | 31 | 11 |
| Smoker at both time-points | 1 | 6 | 4 | 3 | 5 | 9 | 7 | 3 |
| Social mobility | | | | | | | | |
| Persistently low | 3 | 17 | 16 | 13 | 6 | 11 | 40 | 15 |
| Persistently moderate | 3 | 17 | 11 | 9 | 7 | 13 | 30 | 11 |
| Persistently high | 0 | 0 | 3 | 2 | 4 | 7 | 13 | 5 |
| Downwardly mobile | 7 | 39 | 51 | 41 | 26 | 48 | 85 | 32 |
| Upwardly mobile | 5 | 28 | 44 | 35 | 11 | 20 | 100 | 37 |
| Female participants currently using HC | 12 | 46 | 8 | 31 | 12 | 39 | 59 | 32 |
| HDL cholesterol | | | | | | | | |
| Δ BMI | 18 | 0.09 (1.28) | 81 | -0.17 (0.82) | 118 | 0.45 (0.74) | 270 | 0.08 (0.84)† |
| Δ waist circumference | 18 | 0.08 (1.14) | 81 | -0.26 (0.69)* | 118 | 0.49 (0.75) | 271 | 0.01 (0.92)† |
| Δ skin folds (sum of 4) | 18 | -0.05 (1.87) | 80 | -0.24 (0.89) | 118 | 0.51 (0.81) | 268 | 0.05 (0.97)† |
| Δ cardiorespiratory fitness | 13 | 0.06 (1.27) | 66 | 0.19 (1.30) | 95 | -0.22 (1.27) | 224 | 0.08 (1.13)† |
| Δ saturated fat intake§ | 15 | -0.29 (1.92) | 61 | 0.03 (1.42) | 64 | 0.14 (1.42) | 166 | -0.01 (1.33) |
| Smoking status | | | | | | | | |
| Never smoker | 7 | 41 | 68 | 74 | 86 | 80 | 215 | 79 |
| Stopped smoking | 0 | 0 | 9 | 10 | 8 | 7 | 15 | 6 |
| Began smoking | 7 | 41 | 10 | 11* | 13 | 12 | 34 | 12 |
| Smoker at both time-points | 3 | 18 | 5 | 5 | 1 | 1 | 9 | 3 |
| Social mobility | | | | | | | | |
| Persistently low | 1 | 6 | 11 | 12 | 18 | 18 | 36 | 14 |
| Persistently moderate | 2 | 12 | 7 | 8 | 13 | 13 | 31 | 12 |
| Persistently high | 1 | 6 | 1 | 1 | 6 | 6 | 12 | 5 |
| Downwardly mobile | 10 | 59 | 31 | 35 | 43 | 42 | 88 | 33 |
| Upwardly mobile | 3 | 18 | 39 | 44 | 23 | 22 | 96 | 37† |

| Blood lipid or lipoprotein variable<br>Lifestyle variable | True Positives<br>N | <br>mean (SD) or % | False positives<br>N | <br>mean (SD) or % | False negatives<br>N | <br>mean (SD) or % | True negatives<br>N | <br>mean (SD) or % |
|---|---|---|---|---|---|---|---|---|
| Female participants currently using HC | 6 | 46 | 10 | 26 | 3 | 21 | 73 | 36 |
| Triglycerides | | | | | | | | |
| Δ BMI | 12 | 0.32 (1.17) | 188 | 0.07 (0.81) | 37 | 0.53 (0.65) | 252 | 0.10 (0.89)† |
| Δ waist circumference | 12 | 0.38 (1.28) | 188 | 0.02 (0.84) | 37 | 0.55 (0.67) | 253 | 0.04 (0.91)† |
| Δ skin folds (sum of 4) | 12 | -0.15 (1.89) | 187 | 0.09 (0.99) | 36 | 0.56 (0.87) | 251 | -0.07 (0.94)† |
| Δ cardiorespiratory fitness | 10 | -0.21 (1.10) | 147 | -0.02 (1.12) | 32 | 0.25 (1.09) | 211 | 0.02 (1.27) |
| Δ saturated fat intake§ | 8 | 0.79 (1.23) | 155 | 0.03 (1.41) | 20 | 0.41 (1.71) | 124 | -0.16 (1.34) |
| Smoking status | 6 | 55 | 134 | 69 | 32 | 76 | 208 | 84 |
| Never smoker | 0 | 0 | 23 | 12 | 1 | 2 | 8 | 3 |
| Stopped smoking | 3 | 27 | 24 | 12 | 9 | 21 | 28 | 11 |
| Began smoking | 2 | 18 | 12 | 6 | 0 | 0 | 4 | 2 |
| Smoker at both time-points | | | | | | | | |
| Social mobility | | | | | | | | |
| Persistently low | 1 | 10 | 28 | 15 | 6 | 15 | 31 | 13 |
| Persistently moderate | 0 | 0 | 20 | 11 | 5 | 13 | 27 | 11 |
| Persistently high | 0 | 0 | 6 | 3 | 2 | 5 | 12 | 5 |
| Downwardly mobile | 7 | 70 | 80 | 42 | 15 | 39 | 74 | 31 |
| Upwardly mobile | 2 | 20 | 55 | 29 | 11 | 28 | 93 | 39 |
| Female participants currently using HC | 7 | 30 | 7 | 23 | 25 | 61 | 52 | 30† |

*P<0.05 for comparisons between true positives (reference group) and false positives using logistic regression

† P<0.05 for comparisons between false negatives and true negatives (reference group) using logistic regression

Totals for percentages may not add to 100 because of rounding.

Abbreviations: HC = hormonal contraception, SD = standard deviation, Δ = denotes change in z-score (follow-up minus baseline)

Note. True positives = those in the high-risk cluster at both childhood and adulthood

False positives = those in the high-risk cluster at childhood, but not at follow-up

False negatives = those not in the high-risk cluster at childhood, but were in adulthood

True negatives = those not in the high-risk cluster at both childhood and adulthood

*Additional analyses*

Because evidence remained in the adjusted models for effects of waist circumference, cardiorespiratory fitness, beginning smoking, and upward social mobility, a score was created using these variables to examine the effect of the number of improved lifestyle changes on the prevalence of low HDL cholesterol in adulthood (Figure 25). The group who did not improve any lifestyle factor between childhood and adulthood had more than double the prevalence of low HDL cholesterol levels in adulthood compared with the study sample mean (26.2% vs. 11.9%). The prevalence of low HDL cholesterol in those who had improved two lifestyle factors was less than one quarter (2.6% vs. 11.9%) of the study sample prevalence. The mean change in the number of positive lifestyle changes for this score was 1.4 ± 0.8.

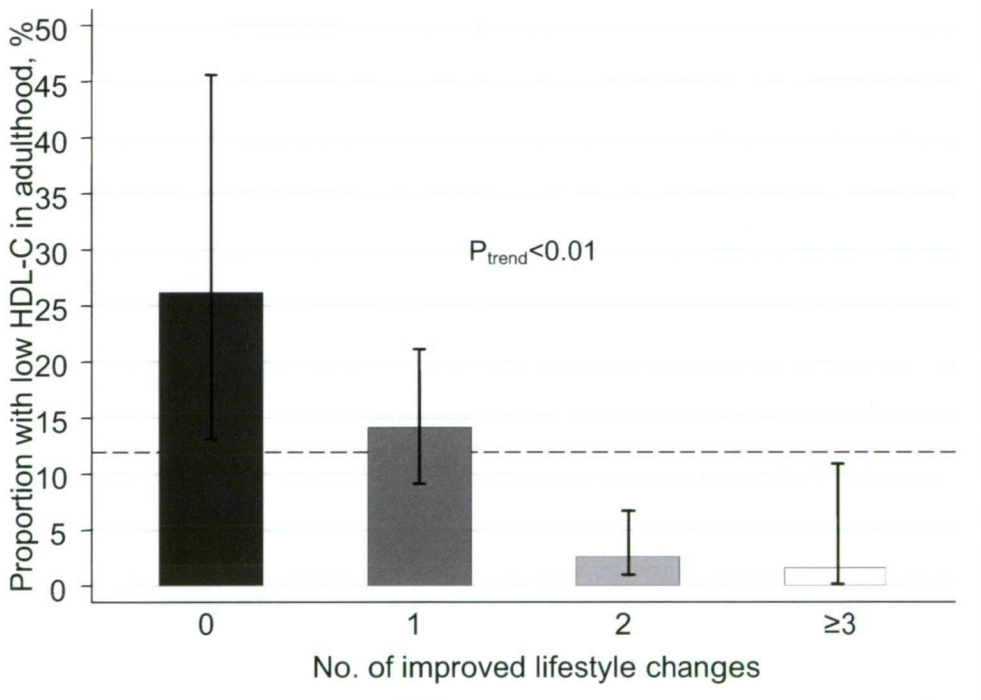


**Figure 25.** Proportion of participants (least squared means) with high-risk (low) HDL cholesterol in adulthood according to the number of positive lifestyle changes from childhood. Positive lifestyle changes included: a ≥1 SD decrease in waist circumference z-score, a ≥1 SD increase in cardiorespiratory fitness z-score, upwardly mobile SEP (as described in methods), and not beginning smoking or not being a persistent smoker. Proportions are adjusted for age and sex. Dashed line indicates population mean of low HDL cholesterol in adulthood. Error bars represent 95% confidence intervals. Number of participants for each group was: 0 changes, N=32; 1 change, N=179; 2 changes, N=120; ≥3 changes, N=39. $P_{trend}$ from logistic model.

## 3.5. DISCUSSION

Previous studies have shown that while tracking of lipid and lipoprotein levels is good, it is not entirely stable. The present study showed that despite rank-order tracking, a substantial proportion of high-risk youth did not have high-risk levels in adulthood, while the majority proportion of those with high-risk levels in adulthood did not have high-risk levels as children or adolescents. These data demonstrated that some of this instability was likely the result of participants adopting changes in lifestyle habits that a screening and intervention program would be designed to promote, such as weight control, physical activity, non-smoking and improvements in socioeconomic circumstance. These findings are important for two reasons. First, they suggest that beneficial changes in potentially modifiable risk factors (smoking and adiposity) in the time between youth and adulthood has the potential to shift those with high-risk blood lipid and lipoprotein levels in childhood or adolescence to not have high-risk levels in adulthood. Second, they emphasise that preventive programs aimed at those who do not have high-risk blood lipid and lipoprotein levels in youth are equally important if the proportion of adults with high-risk levels is to be reduced.

The effects of increased adiposity and beginning smoking on tracking of blood lipid and lipoprotein levels have been suggested in two previous studies. In the six year follow-up of 2236 participants in the Cardiovascular Risk in Young Finns Study, Porkka et al.[223] found that true positives for HDL cholesterol and triglycerides were more likely to increase relative BMI and sum of skin fold measures (bicep, tricep, and subscapular), and to have begun smoking compared with false positives. The reciprocal was also true in comparisons of false negatives and true negatives. Data from 611 and 295 participants from the Beaver County Lipid Study showed that tracking of total cholesterol levels was affected by changes in BMI over 9 year and later 16 year periods.[224, 235] Associations, however, over a longer follow-up period, and with LDL cholesterol reported in this study are novel. Also new are the findings concerning change in cardiorespiratory fitness and social mobility.

These data showed that change in cardiorespiratory fitness was associated with tracking of HDL cholesterol from childhood to adulthood. The beneficial effect of short- to medium-term aerobic exercise training in raising HDL cholesterol levels is well established,[354] with possible underlying mechanisms related to increased lipoprotein lipase activity in adipose tissue and muscle, reductions in the levels of cholesterol ester transfer protein, or a decrease in hepatic lipase activity.[355] The effects of exercise training on HDL cholesterol are thought to be both direct and mediated through exercise-induced reductions in

adiposity.[354] Although not a randomised controlled trial, these data provide observational epidemiological evidence that a long-term increase in cardiorespiratory fitness, and by association, physical activity, is associated with an improved HDL cholesterol profile. The effect was diluted with the inclusion of waist circumference in the multivariable model but remained marginally statistically significant, which provides some support for direct and indirect effects of increased physical activity in raising HDL cholesterol levels.

Although both the Young Finns[223] and Beaver County[224, 235] cohorts examined changes in indices of physical activity collected from questionnaire measurements, neither study found that physical activity significantly affected tracking of the studied blood lipid and lipoprotein variables. The discrepancy between the findings in this study with cardiorespiratory fitness and those from the Young Finns and Beaver County studies that used self-reported physical activity may be a result of reduced measurement error in the objective estimates of cardiorespiratory fitness at both time-points. That is, it would seem possible that self-report physical activity data collected at two time points, using (in some instances) non-validated questions, and delivered to individuals of vastly different ages, would not accurately quantify changes. Because change in cardiorespiratory fitness in an individual is strongly correlated with a change in energy expenditure and measurements are less subject to measurement error, Jackson et al.[356] have previously argued that change in objectively-measured cardiorespiratory fitness from one time-point to another might be a better indicator of change in physical activity than questionnaire measures of physical activity at each time point.

For HDL cholesterol, upward social mobility from childhood to adulthood was associated with improvements in HDL risk status. This finding is consistent with a Scottish study which found that those who moved from a lower SEP in childhood to a higher SEP in adulthood had higher adult HDL cholesterol levels.[357] The mechanisms through which improvements in SEP are related to decreases in HDL cholesterol risk are uncertain. Plausibly, this relationship could be mediated through changes in saturated fat intake, physical activity, smoking or adiposity, but upward social mobility remained independently associated with HDL cholesterol risk status in the multivariable model, which accounted for these and other factors. Possibly, changes in unmeasured variables, such as dietary components other than saturated fat, alcohol consumption, depression, stress or health service utilisation, could explain the observed association.

No association between change in saturated fat intake and blood lipid or lipoprotein tracking was observed. The Beaver County Study found that false positive participants for total cholesterol levels had significantly improved their nutrition score (adherence to a diet low in saturated fat and cholesterol) compared with their false negative counterparts.[224] One other study reporting on cluster tracking of a number of risk factors (total cholesterol, HDL cholesterol, and diastolic blood pressure) has previously reported that false positives significantly decreased their consumption of dietary fat compared with false negatives that increased consumption. Measurement inconsistency in the diet variables used to derive change in saturated fat intake in this study may have contributed to the lack of any association observed between this variable and tracking of the blood lipid and lipoprotein levels. Although related, comparing a direct assessment of saturated fat intake as a percentage of total daily caloric intake in childhood to a measure relating to saturated fat intake behaviour in adulthood is, at best, only a proxy for actual changes in either variable. The data collected at both time points did not allow either fat intake behaviour or saturated fat intake as a percentage of total daily caloric intake to be directly assessed. The resultant measurement error from comparing these related but different indicators of saturated fat intake would likely shift any true effect toward the null.

In the absence of long-term clinical trials, analyses of the type employed in this study are important as they offer insight into the likely effects of changes in modifiable risk factors on whether an individual remains at, or changes risk status between childhood and adulthood. The findings that health promoting lifestyle changes that occur for adiposity, physical activity, smoking, and socioeconomic circumstance between childhood and adulthood have an impact on tracking of blood lipid and lipoprotein levels provides direction for prevention and intervention programs that may have an impact on adult blood lipid and lipoprotein levels and possibly, future CVD risk. While there is a paucity of evidence from clinical trials supporting prevention programs commencing in childhood or adolescence, these data go someway to supporting the recently revised American Academy of Pediatrics[220] statement for the management of hypercholesterolemia in children that endorsed population-wide preventive measures encouraging physical activity and following dietary guidelines both for the reduction in dyslipidemia and for overweight or obesity.

### 3.5.1 LIMITATIONS

Potential bias due to loss to follow-up of almost 70% of the original eligible cohort needs to be considered. Participants at follow-up differed from non-participants with respect to baseline smoking, SEP, and adiposity. The differences in adiposity, although statistically significant, were relatively small in absolute terms. With respect to SEP, sensitivity analyses that stratified tracking by a measure of area-level SEP collected at baseline[348] showed that tracking for those of high SEP at baseline was consistent or lower than that in participants of low, low-medium, or medium-high SEP at baseline (LDL cholesterol: r = 0.57 [high SEP] vs. 0.61 [low, low-medium, or medium-high]; HDL cholesterol: 0.45 vs. 0.47; and triglycerides: 0.31 vs. 0.34 respectively). It is therefore unlikely that the differences in baseline SEP would have biased these results. With respect to smoking, sensitivity analyses that stratified tracking by baseline smoking status showed that tracking correlations were stronger for smokers at baseline compared with non-smokers (LDL cholesterol: r = 0.64 vs. 0.59; HDL cholesterol: 0.56 vs. 0.45; and triglycerides: 0.40 vs. 0.32), suggesting that the tracking estimates presented were likely an underestimate of the true effect.

Several potential sources of measurement error were also evident. First, only single measurements of lipid and lipoprotein levels were recorded at both time points. This was one of the major limitations of most previous studies that have examined tracking of blood lipid and lipoprotein levels from childhood to adulthood that unfortunately remains so for this study. There are considerable data that suggest short- to long-term within-person variability in the measurement of lipids and lipoproteins.[308-310] The variability in blood lipid measures from one time-point to another within the same person is a combination of what Davis et al.[337] defined as preanalytic factors (method of specimen collection), analytic factors (variability of reagents and instrument function), and biological factors (age, pubertal status, diet, season of year, and recency of acute infection). Knowledge of two separate childhood blood lipid values reduces what is known as the regression-toward-the-mean (or regression dilution) effect.[161] Regression toward the mean is a statistical principle that states that persons with low or high measures (e.g. blood lipid) on a first measure will tend to have values closer to the mean of the population distribution (of the blood lipid) on a second measure and will lead to an underestimate of the true effect.[358-360] Davis et al. empirically confirmed this relationship for total cholesterol measurements in predicting CVD risk, showing that a single measure underestimates the true tracking correlation coefficient by ~15% when compared with the average of two measurements and by ~23% when compared with a theoretical error-

free measure.[337] Data from the Bogalusa Heart Study (reproduced in Figure 22) showed that stability tracking for LDL cholesterol increased by 15 percentage points (from 10 to 25%) when two measurements were used to classify levels in youth. What this means for this study is that the degree of tracking would be higher than these results suggested. Several methods to adjust for regression to the mean have been proposed in the literature.[360] However, these methods can not be applied in clinical settings for the prediction of adult blood lipid levels on the basis of childhood levels,[161] which is why the results for this study are not adjusted for regression to the mean. The above however emphasises the importance, from a clinical perspective, of repeat lipid and lipoprotein measurements to decrease the regression to the mean effect and thus assign risk more accurately. Both the current[220] and previous[196, 247] paediatric guidelines for blood lipid screening stipulate multiple measurements before lipid levels are classified.

Second, because data were only available from two time points, only a single measure of change was available that did not allow the timing, duration, or frequency of changes to be examined. Third, measurement inconsistency in the diet variables may have contributed to the lack of any observable association in these data. Other important dietary variables that were unable to be examined include the intakes of cholesterol, polyunsaturated fatty acids, and fibre. It is apparent that other studies are required to answer the question of changes in diet with tracking of blood lipid and lipoprotein levels. Fourth, it was not possible to consider the potential role of pubertal status in these analyses because these data were not collected at baseline. Age was considered in the analyses as a proxy for pubertal status given the relationship between sexual maturation and lipids, and age and lipids have been shown to be similar in adolescents.[300] Fifth, childhood SEP was retrospectively recalled, although this is a commonly used[357, 361] and valid method that does not differ according to SEP.[362] Sixth, the measure of SEP was limited to education; findings may have differed had an alternate indicator been used. Finally, although adverse blood lipid and lipoprotein levels in adulthood are a risk factor for CVD, they do not provide evidence of the outcomes of most interest – CVD and mortality.

## 3.6. CONCLUSIONS

Unhealthy lifestyle changes that occur between childhood or adolescence and adulthood have an impact on whether an individual maintains, loses, or develops high-risk blood lipid and lipoprotein levels in adulthood. These data suggest that prevention and intervention programs designed to promote weight control in the first instance, but also physical activity, non-smoking and improvements in socioeconomic circumstances in the time between youth and adulthood are important for both those with and without high-risk levels in childhood or adolescence.

**KEY POINTS**

- This chapter studied the tracking of total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels over a 20-year period from childhood to adulthood in the Australian Childhood Determinants of Adult Health (CDAH) study, and examined factors that affect the tracking of these blood lipid and lipoprotein levels.

- Tracking (Spearman's rank-order correlations) was observed for total cholesterol ($r=0.54$), low-density lipoprotein cholesterol ($r=0.59$), high-density lipoprotein (HDL) cholesterol ($r=0.47$), and triglycerides ($r=0.34$) (all $P<0.01$).

- Between 40 and 98% of individuals with baseline high-risk blood lipid and lipoprotein levels according to the National Cholesterol Education Program cut-points no longer had high-risk levels at follow-up.

- Participants who acquired high-risk levels in adulthood had significantly greater adiposity gains between surveys compared with those who lost their high-risk status and those who maintained normal-risk levels (All $P\leq0.05$).

- Changes in cardiorespiratory fitness, smoking status and socioeconomic position in addition to waist circumference were also associated with HDL cholesterol tracking ($P\leq0.10$).

- These data suggest that prevention and intervention programs designed to promote weight control in the first instance, but also physical activity, non-smoking and improvements in socioeconomic circumstances in the time between youth and adulthood are important for both those with or without high-risk levels in childhood or adolescence.

**Box 3.** Summary of key points from Chapter 3: factors affecting blood lipid and lipoprotein tracking

# 4. UTILITY OF TWO CLASSIFICATIONS OF PAEDIATRIC DYSLIPIDEMIA TO PREDICT DYSLIPIDEMIA IN ADULTHOOD

## 4.1. INTRODUCTION

As outlined in section 1.6, interest in screening children and adolescents for lipid disorders has gained momentum in recent times, with reports from the US Preventive Service Task Force,[250] the AHA,[249] and others,[220, 251-253] outlining challenges with the existing guidelines issued by NCEP and calling for its revision.[196] One area of the existing guidelines outlined for revision included the lipid and lipoprotein cut-points used to assign risk status. Two classifications have been circulated that provide paediatric cut-points for what constitutes normal-, borderline-, and high-risk levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. The first was a single set of cut-points issued by NCEP,[196] that defined borderline-, and high-risk cut-points at values coinciding with the 75$^{th}$, and 95$^{th}$ percentiles (25$^{th}$, and 5$^{th}$ for HDL cholesterol) of the entire population distribution from the LRC Prevalence Study. The NCEP cut-points apply to all children and adolescents aged 2 to 19 years, hence do not account for changes in lipid distributions that occur with growth and maturation. The second classification derived age- and sex-specific cut-points for adolescents aged 12 to 19 years using data from three NHANES.[251] These cut-points were derived using growth curves that were linked to evidence-based dyslipidemia thresholds for adults. Although these two classifications of adolescent dyslipidemia have been circulated, no study has assessed which of these classifications is most effective for predicting those adolescents who will develop abnormal levels in adulthood.

## 4.2. AIMS

The aims of this chapter were:

1. To determine the utility of two classifications of paediatric dyslipidemia to predict dyslipidemia in adulthood.
2. To evaluate the effect of different screening strategies on the ability to identify adolescents who would develop dyslipidemia as adults.

## 4.3. METHODS

To address these aims, data from the three population-based, prospective cohort studies described in chapter 2 were used.

### 4.3.1 STUDY SAMPLES AND MEASURES

**Australian Data (The Childhood Determinants of Adult Health [CDAH] Study)**

*Study sample*

For these analyses, data from 365 participants (33 % of those eligible from baseline, 49 % male) aged 12 and 15 years at baseline who had lipoprotein data available at both baseline (1985) and follow-up (2004-2006) were used.

*Measures*

Protocols for baseline and follow-up measures of blood lipids and lipoproteins, and height and weight measures have been described in section 2.2.3.

**Finnish Data (The Cardiovascular Risk in Young Finns Study)**

*Study sample*

For the present analyses, data on 1185 subjects (66 % of those eligible from baseline, 45 % male) with lipoprotein data available in 1980 and 2001 and who were aged 12, 15, and 18 years at baseline (in 1980) were included.

*Measures*

Protocols for baseline and follow-up measures of blood lipids and lipoproteins, and height and weight measures have been described in section 2.3.3.

*Family History of Premature Coronary Heart Disease*: Family history of premature coronary heart disease (CHD) was assessed by a questionnaire in 2001. Three different classifications, from stringent to broad, were established to assess family history.[83] Family history was considered positive only if each participant's father *or* mother had: (1) experienced myocardial infarction or had percutaneous coronary intervention or coronary bypass surgery at ≤55 years of age (N = 120); (2) been diagnosed with CHD, experienced myocardial infarction, or had percutaneous coronary intervention or coronary bypass surgery at ≤55 years of age (N = 176); or (3) been diagnosed with CHD, experienced myocardial

infarction, or had percutaneous coronary intervention or coronary bypass surgery at any age (N = 382). Results were essentially similar when using any of these three classifications. Results are expressed using classification two, as has been used to denote positive family history in previous work from the Young Finns study.[83]

**United States Data (The Bogalusa Heart Study)**

*Study sample*

For these analyses, 273 participants (18 % of those eligible from baseline, 44 % male, 29 % African American) aged 12 to 17 years at baseline who had fasting lipid and lipoprotein data available from both baseline (1984-1985) and follow-up (2001-2002) surveys were used.

*Measures*

Protocols for baseline and follow-up measures of blood lipids and lipoproteins, and height and weight measures have been described in section 2.4.3.

### 4.3.2 CLASSIFICATION OF LIPOPROTEIN STATUS IN ADOLESCENCE AND ADULTHOOD

Status of blood lipid and lipoprotein variables in adolescents was defined according to NCEP[196] and NHANES[251] classifications (Table 13). In adulthood, NCEP ATPIII guidelines for total cholesterol (≥6.22 mmol/L; ≥240 mg/dL), LDL cholesterol (≥4.14 mmol/L; ≥160 mg/dL), HDL cholesterol (<1.036 mmol/L; <40 mg/dL), and triglycerides (≥2.26 mmol/L; ≥200 mg/dL) were used to identify those at high risk (i.e. those with substantially increased risk of CVD).[304]

**Table 13.** NCEP[196] and NHANES[251] adolescent lipoprotein cut-points (mmol/L)

| | NCEP | NHANES | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12y | | 13y | | 14y | | 15y | | 16y | | 17y | | 18y | |
| | | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Total cholesterol | | | | | | | | | | | | | | | |
| Normal | <4.40 | <5.18 | <4.77 | <4.99 | <4.71 | <4.86 | <4.68 | <4.84 | <4.72 | <4.88 | <4.82 | <4.95 | <4.94 | <5.05 | <5.07 |
| Borderline | 4.40- | 5.18- | 4.77- | 4.99- | 4.71- | 4.86- | 4.68- | 4.84- | 4.72- | 4.88- | 4.82- | 4.95- | 4.94- | 5.05- | 5.07- |
| high | 5.17 | 6.02 | 5.46 | 5.82 | 5.40 | 5.69 | 5.37 | 5.69 | 5.45 | 5.76 | 5.61 | 5.87 | 5.81 | 6.01 | 6.02 |
| High | ≥5.18 | ≥6.03 | ≥5.47 | ≥5.83 | ≥5.41 | ≥5.70 | ≥5.38 | ≥5.70 | ≥5.46 | ≥5.77 | ≥5.62 | ≥5.88 | ≥5.82 | ≥6.02 | ≥6.03 |
| LDL cholesterol | | | | | | | | | | | | | | | |
| Normal | <2.85 | <2.50 | <2.38 | <2.44 | <2.41 | <2.39 | <2.41 | <2.38 | <2.43 | <2.41 | <2.45 | <2.46 | <2.47 | <2.51 | <2.52 |
| Above | - | 2.50- | 2.38- | 2.44- | 2.41- | 2.39- | 2.41- | 2.38- | 2.43- | 2.41- | 2.45- | 2.46- | 2.47- | 2.51- | 2.52- |
| normal | | 3.23 | 2.95 | 3.14 | 2.97 | 3.07 | 2.99 | 3.05 | 3.02 | 3.10 | 3.06 | 3.17 | 3.12 | 3.24 | 3.21 |
| Borderline | 2.85- | 3.24- | 2.96- | 3.15- | 2.98- | 3.08- | 3.00- | 3.06- | 3.03- | 3.11- | 3.07- | 3.18- | 3.13- | 3.25- | 3.22- |
| high | 3.36 | 3.97 | 3.51 | 3.85 | 3.54 | 3.75 | 3.56 | 3.73 | 3.60 | 3.80 | 3.67 | 3.90 | 3.76 | 3.99 | 3.89 |
| High | ≥3.37 | ≥3.98 | ≥3.52 | ≥3.86 | ≥3.55 | ≥3.76 | ≥3.57 | ≥3.74 | ≥3.61 | ≥3.81 | ≥3.68 | ≥3.91 | ≥3.77 | ≥4.00 | ≥3.90 |
| HDL cholesterol | | | | | | | | | | | | | | | |
| Normal | >1.56 | ≥1.70 | ≥1.48 | ≥1.64 | ≥1.47 | ≥1.59 | ≥1.48 | ≥1.55 | ≥1.49 | ≥1.53 | ≥1.51 | ≥1.53 | ≥1.53 | ≥1.54 | ≥1.54 |
| Borderline | 1.56- | 1.69- | 1.47- | 1.63- | 1.46- | 1.58- | 1.47- | 1.54- | 1.48- | 1.52- | 1.50- | 1.52- | 1.52- | 1.53- | 1.53- |
| low | 0.91 | 1.14 | 1.04 | 1.11 | 1.05 | 1.08 | 1.05 | 1.05 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 |
| Low | <0.91 | ≤1.13 | ≤1.03 | ≤1.10 | ≤1.04 | ≤1.07 | ≤1.04 | ≤1.04 | ≤1.03 | ≤1.03 | ≤1.03 | ≤1.03 | ≤1.03 | ≤1.03 | ≤1.03 |
| Triglycerides | | | | | | | | | | | | | | | |
| Normal | <1.02 | <1.44 | <1.60 | <1.48 | <1.53 | <1.52 | <1.47 | <1.56 | <1.44 | <1.59 | <1.46 | <1.62 | <1.53 | <1.65 | <1.61 |
| Borderline | 1.02- | 1.44- | 1.60- | 1.48- | 1.53- | 1.52- | 1.47- | 1.56- | 1.44- | 1.59- | 1.46- | 1.62- | 1.53- | 1.65- | 1.61- |
| high | 1.46 | 1.83 | 2.02 | 1.92 | 1.92 | 2.01 | 1.81 | 2.09 | 1.78 | 2.15 | 1.82 | 2.19 | 1.93 | 2.23 | 2.08 |
| High | ≥1.47 | ≥1.84 | ≥2.03 | ≥1.93 | ≥1.93 | ≥2.02 | ≥1.82 | ≥2.10 | ≥1.79 | ≥2.16 | ≥1.83 | ≥2.20 | ≥1.94 | ≥2.24 | ≥2.09 |

Abbreviations: y, age in years; M, males; F, females

### 4.3.3 STATISTICAL ANALYSES

Two CDAH participants (one male), five Young Finn's participants (all male), and seven Bogalusa participants (three males) were currently taking lipid lowering medications at follow-up and were removed from the analyses. Including or excluding these participants in the analyses made no difference to the final results presented.

*Comparison of participants and non-participants*

Comparisons between baseline characteristics of participants and non-participants at follow-up within each cohort were performed using logistic regression. Participation (yes/no) was used as the binary dependent variable in these analyses.

*Descriptive analyses*

Descriptive statistics were used to summarise participant characteristics at baseline and follow-up for each cohort. Continuous variables were expressed as means ± SD, while dichotomous variables were presented as proportions.

*Prediction of abnormal lipoprotein levels in adulthood*

*Relative risks*

Log-binomial regression was used to examine associations between baseline lipid and lipoprotein classifications and the development of abnormal levels at follow-up. Relative risks and 95% confidence intervals were calculated for cohort-stratified and pooled data. Estimates were adjusted for age at baseline, sex, and change in BMI rank between adolescence and adulthood as it was associated with adulthood lipid and lipoprotein levels in all cohorts. This adjustment allowed the associations to be examined independent of any change in BMI, which would likely affect the prediction of adult status from adolescent classifications (as was shown in chapter 3). Analyses of Bogalusa data were also adjusted for race; pooled estimates were additionally adjusted for cohort and length of follow-up. The adjustment for cohort was to account for differences in lipid and lipoprotein determination methods between the three cohorts; the adjustment for length of follow-up was to account for any within-cohort differences between length of follow-up and risk of high-risk lipid or lipoprotein levels adulthood. Interactions between cohort and adolescent lipid and lipoprotein classifications were added to each pooled model and examined for significance. There were significant interactions between cohort and NCEP HDL cholesterol cut-points, and cohort

and NHANES HDL cholesterol cut-points. These data are considered in the results. BMI data were not available for 44 Australian, eight Finns, and two US participants at follow-up. Hence, log-binomial analyses were performed on reduced sample sizes of up to 319 CDAH, 1172 Young Finns, and 264 Bogalusa participants.

*Direct comparisons of NCEP vs. NHANES classifications*

The ability of each adolescent classification to predict abnormal adult levels was assessed using diagnostic performance statistics, including: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under receiver-operating characteristic curves (AUC). Sensitivity was calculated as true positives/(true positives + false negatives), specificity as true negatives/(true negatives + false positives), PPV as true positives/(true positives + false positives), and NPV as true negatives/(true negatives + false negatives). Tests for significant differences of sensitivity and specificity between NCEP and NHANES adolescent cut-points were performed using McNemar's test.[363] Confidence intervals for sensitivity and specificity were calculated using the binomial distribution. The AUC has a range of 0 to 1, where a value of 0.5 represents no discrimination, and a value of 1 would indicate perfect discrimination. Tests for significant differences between AUC for each adolescent classification were calculated using the DeLong algorithm.[364] This method assumes the correct null distribution when there are only three classification levels as is the case here (confirmed through simulation).

Point estimates for pooled data are presented in the results, with cohort-stratified data presented graphically. Log binomial regression analyses and diagnostic performance statistics were calculated twice for each data set: the first using NCEP cut-points, and the second using NHANES cut-points.

*Evaluation of different screening strategies*

To evaluate whether different screening strategies had an effect on the ability to identify adolescents who would develop associated dyslipidemia as adults, the existing NCEP paediatric screening algorithm was considered that uses positive family history as a criterion before children and adolescents are subject to lipid and lipoprotein analysis.[196] The efficacy of lipid screening in adolescents who were overweight or obese in accordance with recent recommendations by the AHA and the AAP was also examined.[220, 249] The Young Finns cohort was chosen for these analyses to maximise sample numbers and take advantage of comprehensive data on family history of premature CHD available from the 2001 follow-up

survey. To identify adolescent Finns who may develop high-risk levels as adults, the following screening strategies were employed: (a) universal (whole- or random-population) screening employing the best performing high-risk cut-points from earlier analyses, (b) positive family history of CHD and lipid or lipoprotein levels exceeding best-performing high-risk cut-points, (c) overweight or obesity according to International Obesity Task Force criteria[306] and lipid or lipoprotein levels exceeding best-performing high-risk cut-points, and (d) positive family history of CHD *or* overweight or obesity and lipid or lipoprotein levels exceeding best-performing high-risk cut-points. Sensitivity, specificity, PPV, NPV, and AUC were calculated for each screening strategy.

## 4.4. RESULTS

To determine if the participants at follow-up were representative of the original eligible cohorts, baseline characteristics between participants and those lost to follow-up were compared (Table 14). Those lost to follow-up (67 % CDAH, 34 % Young Finns, 82 % Bogalusa) were more likely to be younger and male in all cohorts, and African American in the Bogalusa cohort (all $P<0.05$), but there were no statistically or clinically significant differences at baseline between participants and non-participants in any of the three cohorts in total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels in age- and sex-adjusted analyses.

**Table 14.** Characteristics at baseline of participants and non-participants at follow-up

| | CDAH | | Young Finns | | Bogalusa | |
|---|---|---|---|---|---|---|
| | Participants | Non-participants | Participants | Non-participants | Participants | Non-participants |
| N | 365 | 744 | 1185 | 601 | 273 | 1239 |
| Age at baseline, y* | 13.5 | 13.3§ | 14.9 | 14.6§ | 15.3 | 14.6§ |
| Males, %† | 49.0 | 56.6§ | 44.6 | 55.9§ | 44.0 | 52.1§ |
| African American, %‡ | - | - | - | - | 29.3 | 36.8§ |
| Total cholesterol, mmol/L‡ | 4.44 | 4.44 | 5.18 | 5.17 | 4.16 | 4.15 |
| LDL cholesterol, mmol/L‡ | 2.68 | 2.68 | 3.30 | 3.30 | 2.46 | 2.42 |
| HDL cholesterol, mmol/L‡ | 1.43 | 1.41 | 1.55 | 1.54 | 1.49 | 1.57 |
| Triglycerides, mmol/L‡ | 0.76 | 0.79 | 0.73 | 0.72 | 0.88 | 0.81 |

Statistics are means for continuous variables and percentages for dichotomous variables

*Differences between participants and non-participants examined using logistic regression adjusted for sex (additionally adjusted for race in Bogalusa analyses).

†Differences between participants and non-participants examined using logistic regression adjusted for age (additionally adjusted for race in Bogalusa analyses).

‡Differences between participants and non-participants examined using logistic regression adjusted for sex and age (additionally adjusted for race in Bogalusa analyses).

§$P<0.05$

### 4.4.1 ADOLESCENT AND ADULT LIPID AND LIPOPROTEIN LEVELS

Adolescent and adult lipid and lipoprotein levels, and the proportion of adults classified with lipid disorders in each study cohort are displayed in Table 15. With the exception of HDL cholesterol, females tended to have a worse lipid and lipoprotein profile in adolescence compared with their male counterparts, but this trend was reversed in adulthood. Compared with adult females, a higher proportion of adult males were classified as having abnormal levels of LDL cholesterol, HDL cholesterol, and triglycerides across all cohorts.

**Table 15.** Levels of lipid and lipoprotein variables in adolescence and adulthood in cohorts from Australia, Finland, and the United States

| | Males | | Females | |
|---|---|---|---|---|
| | Adolescence | Adulthood | Adolescence | Adulthood |
| CDAH (N=363) | | | | |
| N | 178 | | 185 | |
| Age, y | 13.5±1.5 | 33.4±1.7 | 13.5±1.5 | 33.4±1.6 |
| Total cholesterol, mmol/L | 4.33±0.68 | 5.11±0.95 | 4.52±0.71 | 4.85±0.95 |
| LDL cholesterol, mmol/L | 2.61±0.60 | 3.23±0.78 | 2.71±0.66 | 2.84±0.79 |
| HDL cholesterol, mmol/L | 1.39±0.28 | 1.30±0.29 | 1.47±0.27 | 1.56±0.35 |
| Triglycerides, mmol/L | 0.75±0.34 | 1.30±0.81 | 0.76±0.34 | 0.97±0.58 |
| High total cholesterol* | | 13.5 | | 6.5 |
| High LDL cholesterol* | | 11.4 | | 4.3 |
| Low HDL cholesterol* | | 22.5 | | 7.0 |
| High triglycerides* | | 10.1 | | 4.9 |
| Young Finns (N=1180) | | | | |
| N | 523 | | 657 | |
| Age, y | 14.9±2.4 | 35.9±2.4 | 14.8±2.4 | 35.8±2.4 |
| Total cholesterol, mmol/L | 5.07±0.87 | 5.52±1.01 | 5.27±0.87 | 5.18±0.95 |
| LDL cholesterol, mmol/L | 3.24±0.79 | 3.65±0.91 | 3.35±0.79 | 3.27±0.79 |
| HDL cholesterol, mmol/L | 1.50±0.31 | 1.18±0.29 | 1.58±0.29 | 1.40±0.30 |
| Triglycerides, mmol/L | 0.70±0.33 | 1.62±1.09 | 0.75±0.34 | 1.17±0.75 |
| High total cholesterol* | | 21.4 | | 12.9 |
| High LDL cholesterol* | | 27.0 | | 12.2 |
| Low HDL cholesterol* | | 33.9 | | 11.1 |
| High triglycerides* | | 17.6 | | 5.6 |
| Bogalusa (N=266) | | | | |
| N | 117 | | 149 | |
| Age, y | 15.3±1.6 | 32.4±1.4 | 15.3±1.5 | 32.3±1.5 |
| Total cholesterol, mmol/L | 4.05±0.88 | 4.89±0.94 | 4.20±0.77 | 4.74±0.99 |
| LDL cholesterol, mmol/L | 2.38±0.71 | 3.24±0.79 | 2.47±0.59 | 3.03±0.87 |
| HDL cholesterol, mmol/L | 1.43±0.53 | 1.10±0.29 | 1.55±0.47 | 1.29±0.37 |
| Triglycerides, mmol/L | 0.90±0.48 | 1.66±1.46 | 0.82±0.37 | 1.16±0.57 |
| High total cholesterol* | | 6.8 | | 8.7 |
| High LDL cholesterol* | | 15.4 | | 12.1 |
| Low HDL cholesterol* | | 53.0 | | 24.2 |
| High triglycerides* | | 12.8 | | 4.7 |

Data are means ± SD for continuous variables or percent for dichotomous variables.

* Criteria for each adult lipoprotein cut-point are described in Methods.

To convert total cholesterol, LDL cholesterol and HDL cholesterol to mg/dl, multiply values by 38.67; to convert triglyceride values to mg/dl, multiply values by 88.5.

Table 16 shows differences in lipid and lipoprotein levels between 15 year old male and female participants from each cohort. Observations were restricted to those aged 15 years for these analyses because lipid and lipoprotein levels differ by age in adolescence, and the 15 year age group was represented in each cohort and provided adequate numbers for comparison (participants aged 12 years were also represented in each cohort but the sample size in these strata for the Bogalusa study was low). Young Finns participants had higher total and LDL cholesterol concentrations compared with CDAH or Bogalusa participants. CDAH participants had the lowest HDL cholesterol concentrations, while Bogalusa participants had the highest triglyceride levels across the three study sites. These findings need to be considered in terms of differences in lipid and lipoprotein measurement methods between each of the cohorts and is considered in the discussion.

**Table 16.** Levels of lipid and lipoprotein variables in 15 year old male and female adolescents in cohorts from Australia, Finland, and the United States

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Total cholesterol, mmol/L | LDL cholesterol, mmol/L | HDL cholesterol, mmol/L | Triglycerides, mmol/L | N | Total cholesterol, mmol/L | LDL cholesterol, mmol/L | HDL cholesterol, mmol/L | Triglycerides, mmol/L |
| CDAH | 89 | 4.13±0.62 | 2.47±0.57 | 1.31±0.26 | 0.79±0.35 | 92 | 4.40±0.67 | 2.57±0.61 | 1.47±0.28 | 0.79±0.39 |
| Young Finns | 182 | 4.87±0.81* | 3.11±0.72* | 1.43±0.72* | 0.72±0.34 | 218 | 5.19±0.85* | 3.26±0.81* | 1.60±0.31* | 0.72±0.30 |
| Bogalusa | 25 | 3.83±0.78† | 2.17±0.66*† | 1.48±0.40* | 0.86±0.51 | 36 | 4.04±0.76*† | 2.43±0.61† | 1.56±0.54 | 0.82±0.34 |

* Differences between Young Finns or Bogalusa participants and CDAH participants (linear regression, $P<0.05$).

†Differences between Bogalusa and Young Finns participants (linear regression, $P<0.05$).

Note. Triglycerides were log transformed before linear regression was conducted owing to a right-skewed distribution.

To convert total cholesterol, LDL cholesterol and HDL cholesterol to mg/dl, multiply values by 38.67; to convert triglyceride values to mg/dl, multiply values by 88.5.

The proportions of adolescents within normal-, borderline-, and high-risk categories according to NCEP and NHANES classifications are provided in Table 17. The proportions of adolescents with high-risk levels who had dyslipidemia in adulthood were higher for the NCEP cut-point compared with NHANES for total cholesterol, LDL cholesterol, and triglycerides. Whereas, the proportions of participants who had low HDL at both time points was higher when adolescent HDL cholesterol levels were classified using the NHANES cut-point when compared with the NCEP cut-point. While these trends are consistent within the cohorts, the proportions of participants who were stable in the high-risk category varied substantially between cohorts. For example, the proportions of males and females who had high-risk LDL cholesterol levels (according to the NCEP adolescent classification) at both time points ranged from 8.6 to 19.0% for the CDAH and Bogalusa studies, while the proportions who were stable in these groups were 38.5% for male Finns and 43.8% for female Finns.

The analyses presented in Table 17 are analogous to stability tracking. The results show that total cholesterol dyslipidemia persisted into adulthood for 11 to 49% of adolescents using the NCEP classification, and 2 to 31% of adolescents using the NHANES classification. LDL cholesterol dyslipidemia persisted into adulthood for 11 to 44% of adolescents using the NCEP classification, and 2 to 31% of adolescents using the NHANES classification. HDL cholesterol dyslipidemia persisted into adulthood for 0.5 to 15% of adolescents using the NCEP classification, and 2 to 21% of adolescents using the NHANES classification. Triglyceride dyslipidemia persisted into adulthood for 4 to 11% of adolescents using the NCEP classification, and 0.2 to 3% of adolescents using the NHANES classification.

**Table 17**. Proportions of Australian, Finnish, and US adolescents within normal-, borderline-, and high-risk categories according to NCEP and NHANES cut-points for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides

| | CDAH | | | | Young Finns | | | | Bogalusa | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NCEP | | NHANES | | NCEP | | NHANES | | NCEP | | NHANES | |
| | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females | Males | Females |
| Total cholesterol | | | | | | | | | | | | |
| Normal | 53.6 | 46.2 | 85.5 | 64.5 | 23.1 | 15.4 | 52.3 | 35.6 | 71.7 | 66.7 | 85.8 | 78.4 |
| Borderline-high | 35.2 | 34.4 | 12.9 | 25.8 | 35.4 | 35.3 | 32.4 | 33.2 | 15.8 | 20.3 | 9.2 | 13.7 |
| High | 11.2 | 19.4 | 1.7 | 9.7 | 41.5 | 49.3 | 15.3 | 31.2 | 12.5 | 13.1 | 5.0 | 7.8 |
| LDL cholesterol | | | | | | | | | | | | |
| Normal | 66.9 | 61.4 | 41.0 | 38.6 | 33.0 | 29.4 | 14.0 | 12.2 | 79.7 | 79.6 | 59.3 | 47.4 |
| Above normal | - | - | 41.0 | 29.9 | - | - | 36.9 | 27.9 | - | - | 24.6 | 38.2 |
| Borderline-high | 21.9 | 19.6 | 16.3 | 19.6 | 28.6 | 26.8 | 29.7 | 29.1 | 9.3 | 11.8 | 11.0 | 7.9 |
| High | 11.2 | 19.0 | 1.7 | 12.0 | 38.5 | 43.8 | 19.3 | 30.9 | 11.0 | 8.6 | 5.1 | 6.6 |
| HDL cholesterol | | | | | | | | | | | | |
| Normal | 25.8 | 32.1 | 18.0 | 42.4 | 38.3 | 50.7 | 33.0 | 60.0 | 30.0 | 45.1 | 32.5 | 53.6 |
| Borderline-low | 72.5 | 66.9 | 68.0 | 53.3 | 60.2 | 48.9 | 61.9 | 38.4 | 55.0 | 47.7 | 46.7 | 32.7 |
| Low | 1.7 | 1.1 | 14.0 | 4.4 | 1.5 | 0.5 | 5.1 | 1.8 | 15.0 | 7.2 | 20.8 | 13.7 |
| Triglycerides | | | | | | | | | | | | |
| Normal | 86.6 | 83.9 | 95.5 | 96.2 | 84.3 | 84.5 | 97.5 | 96.4 | 68.3 | 73.9 | 90.8 | 92.8 |
| Borderline-high | 8.9 | 11.3 | 3.9 | 2.2 | 11.9 | 11.6 | 2.3 | 2.9 | 20.8 | 17.7 | 5.8 | 4.6 |
| High | 4.5 | 4.8 | 0.6 | 1.6 | 3.8 | 4.0 | 0.2 | 0.8 | 10.8 | 8.5 | 3.3 | 2.6 |

Data presented as percentages, totals may not add to 100 because of rounding.

### 4.4.2 PREDICTION OF ABNORMAL LEVELS OF LIPOPROTEIN VARIABLES IN ADULTHOOD

*Relative risks*

Adjusted relative risks for developing abnormal lipoprotein levels in adulthood across NCEP and NHANES total cholesterol (Figure 26), LDL cholesterol (Figure 27), HDL cholesterol (Figure 28), and triglycerides (Figure 29) cut-points are displayed, along with point-estimates from pooled data in Table 18. The pooled data showed that the risk of developing an abnormal condition in adulthood was significantly higher in those adolescents with borderline- and high-risk levels compared with those with normal levels for all lipoprotein variables. Moreover, a graded increase in the risk of developing abnormal levels in adulthood was observed when moving from the normal, to borderline-risk, to high-risk groups. With the exception of the NHANES high triglyceride threshold in the CDAH cohort (that had no adolescent cases develop the risk factor in adulthood, Figure 29), stratified analyses indicated similar relative risk patterns for borderline- and high-risk classified adolescents in each cohort. Interaction terms between cohort and both NCEP and NHANES HDL cholesterol cut-points were significant, suggesting that the relationship between adolescent risk-status and the development of abnormal HDL cholesterol levels as adults, differed between cohorts. The pooled estimates for HDL cholesterol are included for completeness, however in light of the significant interaction; the pooled estimates should be interpreted with caution. Instead, interpretations are best made using the relative risks stratified by cohort (Figure 28). In cohort stratified analyses, it is evident that the relative risks for HDL cholesterol in the Bogalusa data were substantially lower compared with estimates from CDAH and Young Finns data (Figure 28); race stratified data indicated that the HDL cholesterol classifications predicted abnormal levels in adulthood for African Americans but not Caucasian Americans (Figure 30).

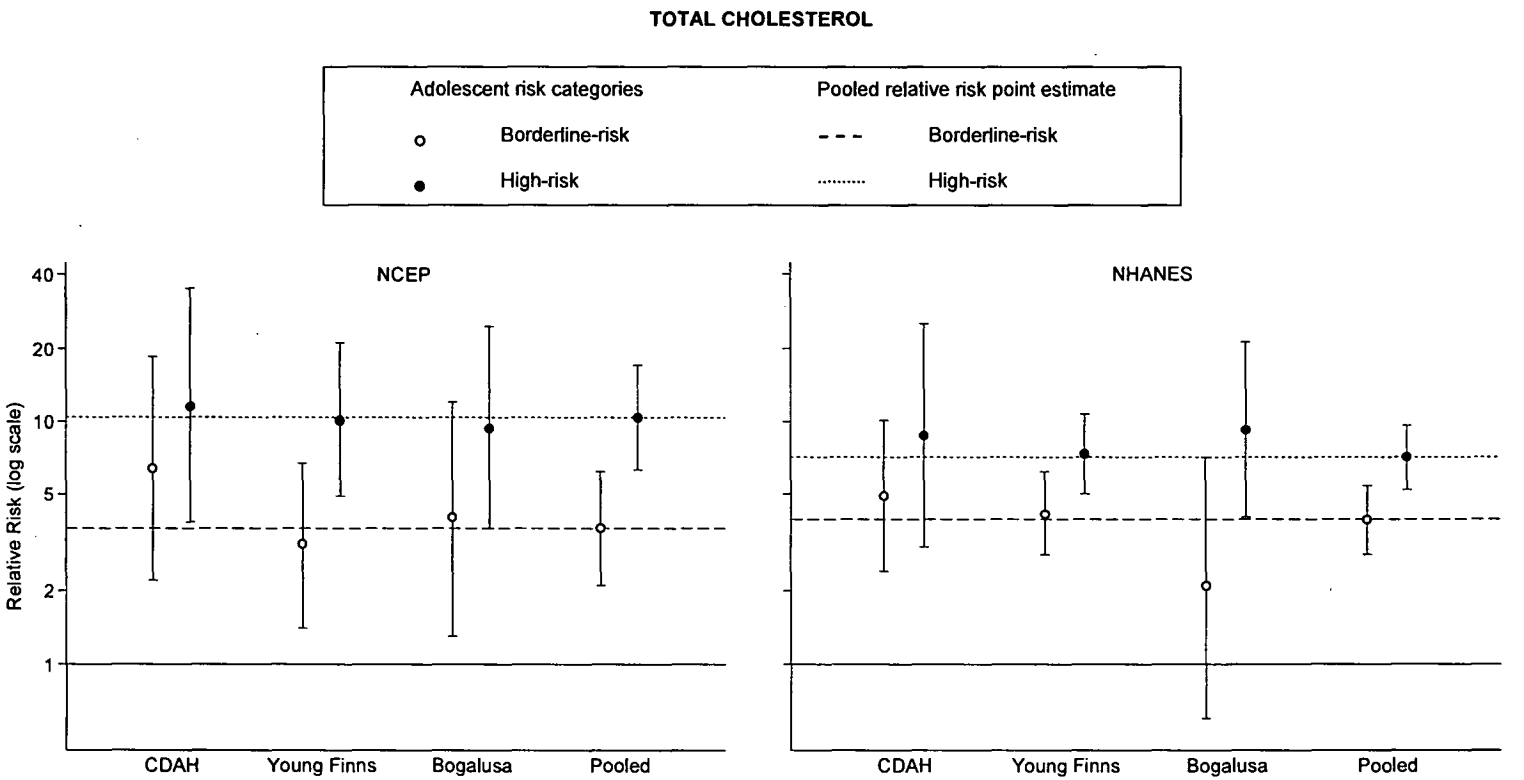


**Figure 26.** Relative risks and 95% confidence intervals of developing abnormal total cholesterol levels in adulthood according to borderline- and high-risk total cholesterol status in adolescence using NCEP and NHANES classifications. Estimates were adjusted for age at baseline, sex, change in BMI rank between adolescence and adulthood; Bogalusa analyses were additionally adjusted for race; estimates from Pooled data were additionally adjusted for cohort and length of follow-up. Normal classified adolescents were used as the referent group. All $P<0.01$ (analysis for trend) unless otherwise noted.

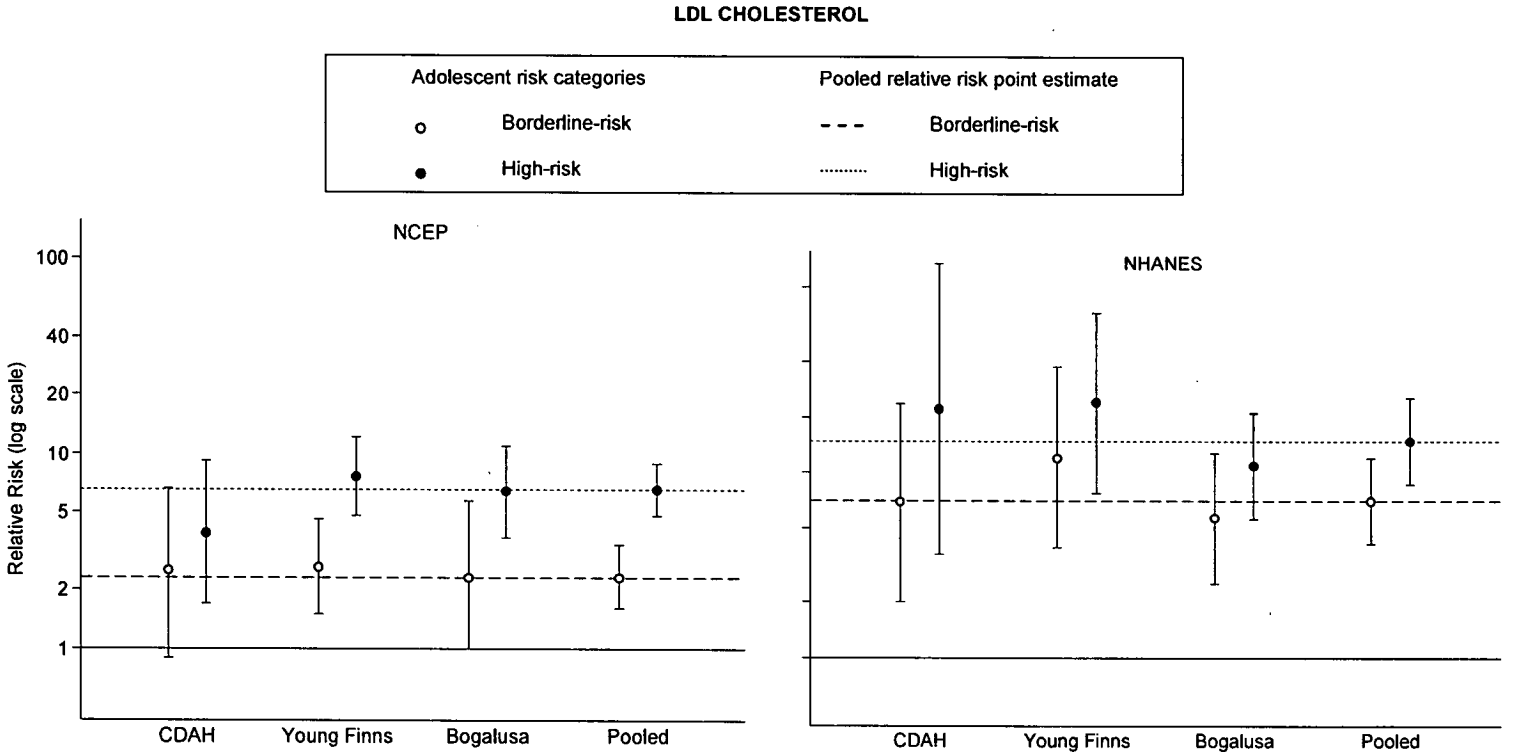


**Figure 27.** Relative risks and 95% confidence intervals of developing abnormal LDL cholesterol levels in adulthood according to borderline- and high-risk LDL cholesterol status in adolescence using NCEP and NHANES classifications. Estimates were adjusted for age at baseline, sex, change in BMI rank between adolescence and adulthood; Bogalusa analyses were additionally adjusted for race; estimates from Pooled data were additionally adjusted for cohort and length of follow-up. Normal classified adolescents were used as the referent group. All $P<0.01$ (analysis for trend) unless otherwise noted.

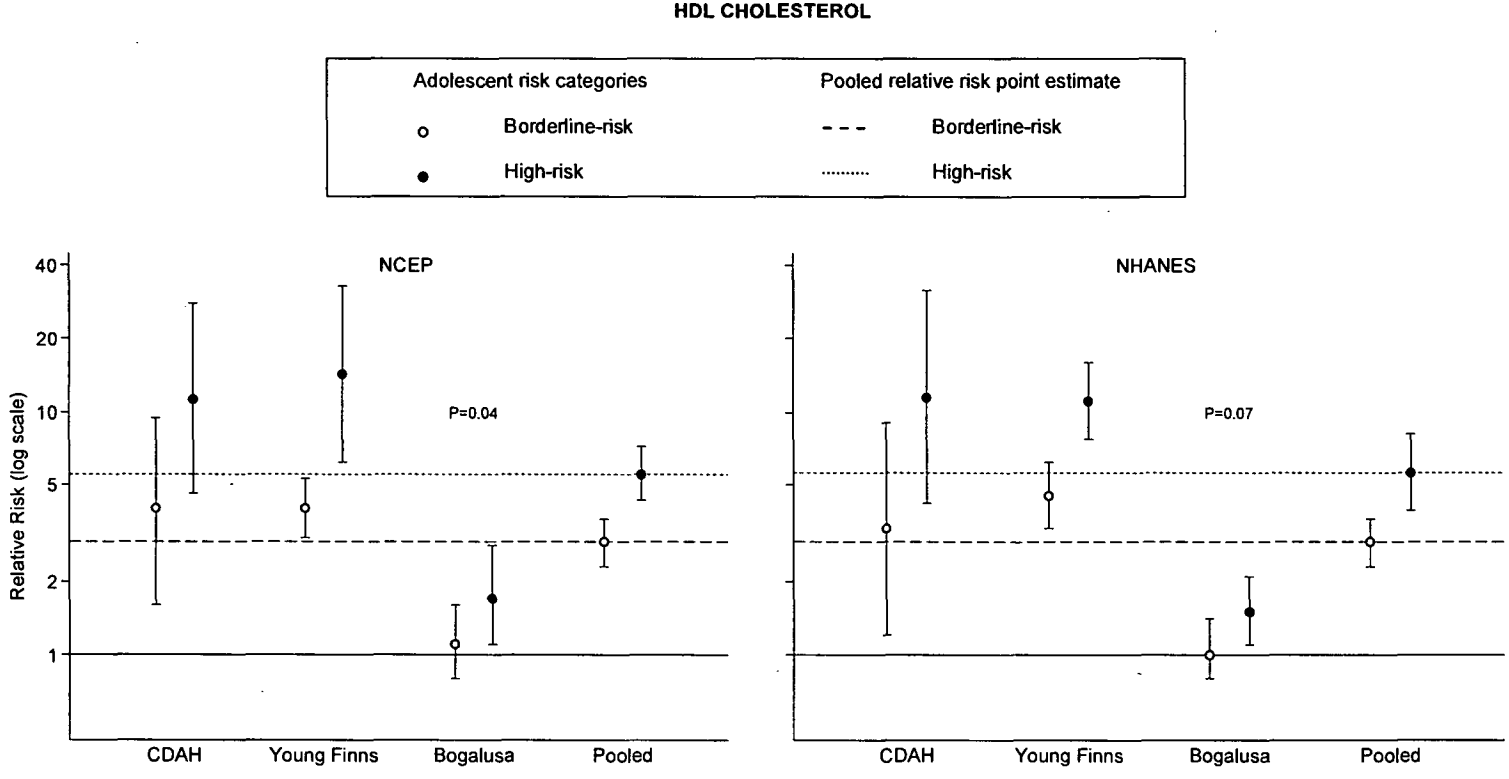


**Figure 28.** Relative risks and 95% confidence intervals of developing abnormal HDL cholesterol levels in adulthood according to borderline- and high-risk HDL cholesterol status in adolescence using NCEP and NHANES classifications. Estimates were adjusted for age at baseline, sex, change in BMI rank between adolescence and adulthood; Bogalusa analyses were additionally adjusted for race; estimates from Pooled data were additionally adjusted for cohort and length of follow-up. Normal classified adolescents were used as the referent group. All P<0.01 (analysis for trend) unless otherwise noted.

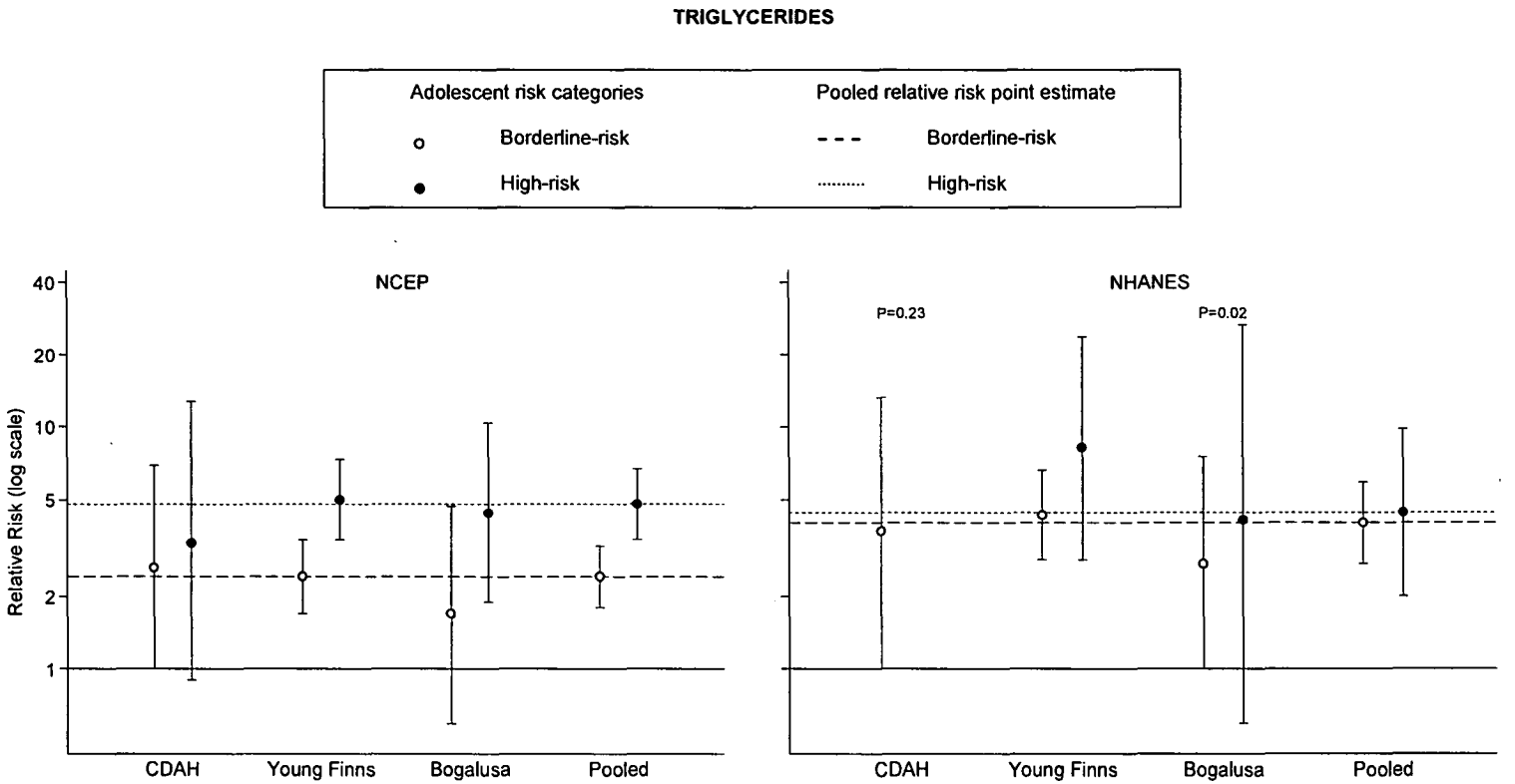


**Figure 29.** Relative risks and 95% confidence intervals of developing abnormal triglyceride levels in adulthood according to borderline- and high-risk triglyceride status in adolescence using NCEP and NHANES classifications. Estimates were adjusted for age at baseline, sex, change in BMI rank between adolescence and adulthood; Bogalusa analyses were additionally adjusted for race; estimates from Pooled data were additionally adjusted for cohort and length of follow-up. Normal classified adolescents were used as the referent group. All P<0.01 (analysis for trend) unless otherwise noted.

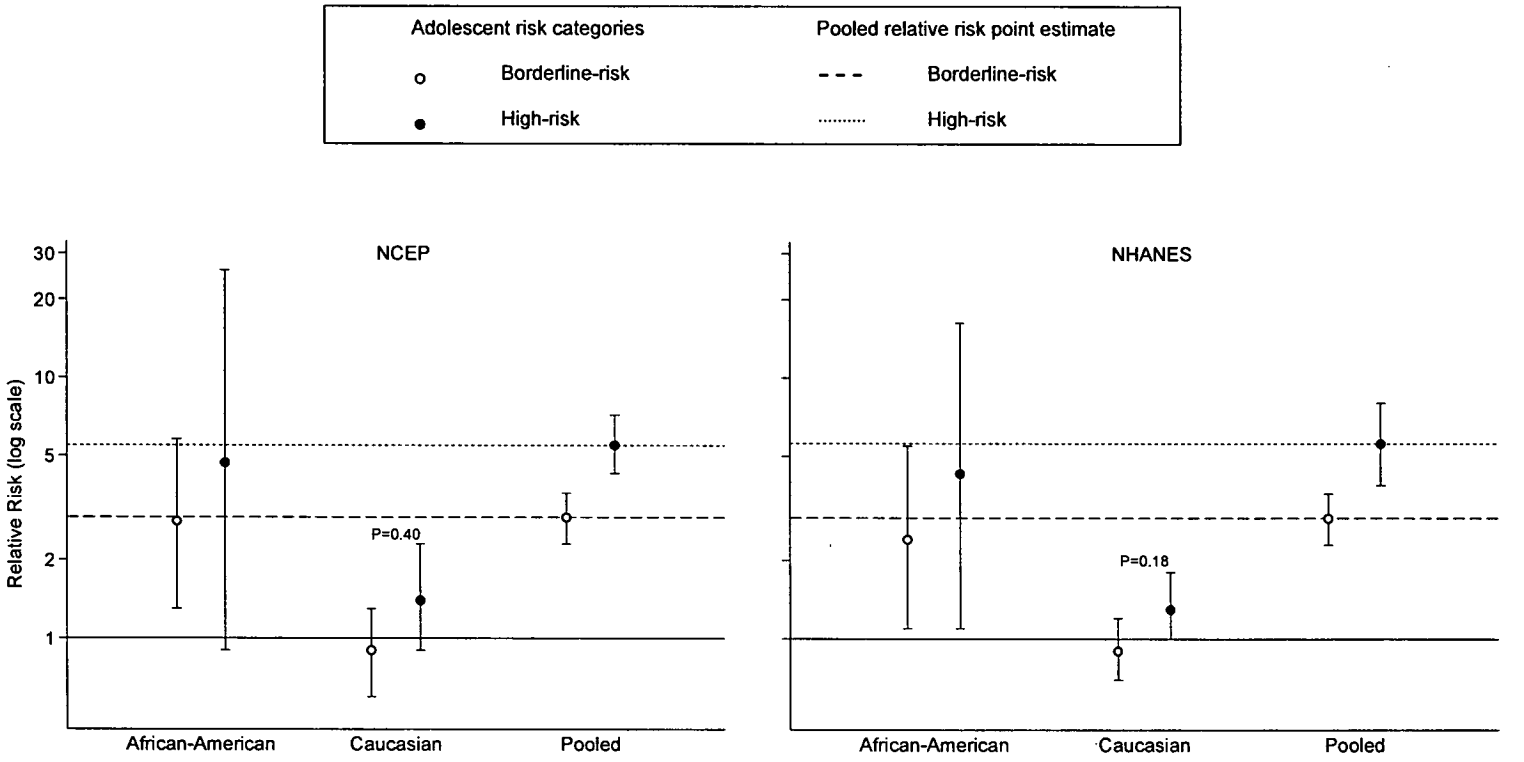


**Figure 30.** Race-stratified US and pooled relative risks and 95% confidence intervals for the development of abnormal HDL cholesterol levels in adulthood according to borderline- and high-risk lipoprotein status in adolescence using NCEP and NHANES classifications. Normal classified adolescents were used as the referent group. All P<0.01 (analysis for trend) unless otherwise noted.

**Table 18.** Relative risks from pooled data for the development of abnormal lipoprotein variable levels in adulthood for those exceeding NCEP and NHANES adolescent lipoprotein cut-points*

| | NCEP | | | NHANES | | |
|---|---|---|---|---|---|---|
| Adolescent cut-points | n/N | RR | 95%CI | n/N | RR | 95%CI |
| Total cholesterol | | | | | | |
| Normal | 17/565 | 1.0 | ref | 53/966 | 1.0 | ref |
| Borderline-high | 57/578 | 3.6 | 2.1-6.2 | 91/471 | 3.9 | 2.8-5.4 |
| High | 169/612 | 10.4 | 6.3-17.1 | 99/318 | 7.1 | 5.2-9.6 |
| LDL cholesterol | | | | | | |
| Normal | 45/774 | 1.0 | ref | 15/414 | 1.0 | ref |
| Above normal | | | | 50/566 | 2.7 | 1.6-4.8 |
| Borderline-high | 49/407 | 2.3 | 1.6-3.4 | 84/415 | 7.0 | 4.1-12.0 |
| High | 179/544 | 6.5 | 4.8-8.9 | 124/330 | 14.8 | 8.6-25.2 |
| HDL cholesterol† | | | | | | |
| Normal | 79/732 | 1.0 | ref | 77/783 | 1.0 | ref |
| Borderline-low | 293/979 | 2.9 | 2.3-3.6 | 255/859 | 2.9 | 2.3-3.6 |
| Low | 27/40 | 5.5 | 4.3-7.2 | 67/109 | 5.6 | 3.9-8.1 |
| Triglycerides | | | | | | |
| Normal | 109/1457 | 1.0 | ref | 151/1689 | 1.0 | ref |
| Borderline-high | 38/219 | 2.4 | 1.8-3.2 | 16/52 | 4.0 | 2.7-5.9 |
| High | 24/79 | 4.8 | 3.4-6.7 | 4/14 | 4.4 | 2.0-9.8 |

* Adjusted for age at baseline, sex, change in BMI rank between adolescence and adulthood, cohort, and length of follow-up. † Interpretation of the pooled relative risks for HDL cholesterol are to be treated with caution as the relative risks significantly differ between cohorts (see Figure 28).

Abbreviations: RR, relative risk; CI, confidence interval

*Direct comparisons of NCEP vs. NHANES classifications*

Diagnostic performance statistics of NCEP and NHANES adolescent cut-points in the pooled data are presented in Table 19. Cohort-stratified and pooled data for sensitivity and specificity of high-risk cut-points are presented for total cholesterol (Figure 31), LDL cholesterol (Figure 32), HDL cholesterol (Figure 33), and triglycerides (Figure 34). Where sample sizes permitted, sex-, age-, or race- (Bogalusa) stratified analyses were comparable with the results presented. Borderline- and high-risk NCEP cut-points for total cholesterol were considerably more sensitive than the corresponding NHANES cut-points, with modest to high tradeoffs in specificity. Although this trend was consistent across each cohort, there was substantial heterogeneity in the sensitivity point estimates and confidence intervals between studies (Figure 31). Of those adults with abnormal total cholesterol levels, 32.3% would not be identified in adolescence using the high-risk NCEP cut-point and 60.6% would not be identified in adolescence using the equivalent NHANES cut-point. The proportion classified as high-risk during adolescence that did not develop the risk factor in adulthood (false positives) was 72.4% for NCEP and 68.7% for NHANES classifications.

The NCEP borderline- and high-risk LDL cholesterol cut-points were more sensitive and less specific than the NHANES cut-points (Table 19, Figure 32). The trade-off in sensitivity-gain and specificity-loss between both classifications was particularly noticeable at the high-risk cut-point. The NHANES high-risk cut-point did not identify 55.4% of those adults with an abnormal LDL cholesterol concentration, as opposed to the NCEP cut-point that did not identify 35.0%. A small improvement in PPV was observed when NHANES vs. NCEP high-risk cut-points were compared. Considerable heterogeneity was observed in sensitivities when cohort-stratified plots were compared.

For HDL cholesterol, the best combination of diagnostic performance statistics was produced when concentrations were classified by the NHANES borderline- and high-risk cut-points (Table 19, Figure 33). Even though sensitivity of the NHANES high-risk cut-point was higher than the corresponding NCEP cut-point, both classifications performed relatively poorly. That is, 83.3% and 93.3% of the adults with low HDL cholesterol would not be identified using the NHANES and NCEP high-risk cut-point respectively. The PPV for the high-risk NCEP cut-point was higher than the NHANES cut-point. Evaluation of AUC indicated that NHANES cut-points were significantly better at predicting low HDL in adulthood compared with NCEP cut-points. Increased sensitivity of the high-risk cut-point

and gains in specificity at the borderline-risk cut-point for NHANES compared with NCEP classifications is likely to explain these differences.

The NCEP classification for triglycerides was a better predictor of high triglyceride levels in adulthood compared with the NHANES classification. For both borderline- and high-risk cut-points, gains in sensitivity were relatively large compared to modest trade-offs in specificity (Table 19, Figure 34). In practical terms however, the sensitivities of both classifications were poor. The classifications did not identify 86.0% (NCEP) and 97.7% (NHANES) of adults with a high triglyceride level. Significantly higher AUC is a reflection of increased sensitivity across all NCEP cut-points.

**Table 19.** Diagnostic performance statistics from pooled data of adolescent borderline- and high-risk lipoprotein variable cut-points, determined using NCEP and NHANES classifications, to predict dyslipidemia in adulthood

| | | Dyslipidemia status in adolescence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Borderline risk | | | | High risk | | | | |
| Lipoprotein variable | Classification | Sensitivity | Specificity | PPV | NPV | Sensitivity | Specificity | PPV | NPV | AUC |
| Total cholesterol | | | | | | | | | | |
| | NCEP | 92.1* | 36.8 | 19.2 | 96.6 | 67.7* | 71.0 | 27.6 | 93.1 | 0.73 |
| | NHANES | 76.4 | 60.6* | 24.1 | 94.0 | 39.4 | 85.9* | 31.3 | 89.7 | 0.71 |
| LDL cholesterol | | | | | | | | | | |
| | NCEP | 82.9* | 50.5 | 23.8 | 94.0 | 65.0* | 75.2 | 32.9 | 92.0 | 0.73 |
| | NHANES | 75.7 | 63.2* | 27.8 | 93.3 | 44.6 | 86.0* | 37.3 | 89.3 | 0.74 |
| HDL cholesterol | | | | | | | | | | |
| | NCEP | 80.3 | 47.4 | 30.3 | 89.4 | 6.7 | 99.0* | 65.9 | 78.8 | 0.65 |
| | NHANES | 80.8 | 51.3* | 32.1 | 90.3 | 16.7* | 96.7 | 59.3 | 80.3 | 0.69† |
| Triglycerides | | | | | | | | | | |
| | NCEP | 35.4* | 84.9 | 20.4 | 92.3 | 14.0* | 96.5 | 30.5 | 91.1 | 0.61† |
| | NHANES | 11.2 | 97.1* | 29.4 | 90.9 | 2.3 | 99.3* | 26.7 | 90.3 | 0.54 |

* $P<0.05$, McNemar's test for difference between sensitivities/specificities; † $P<0.05$, test for difference between AUCs.

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the receiver-operating characteristic curve.

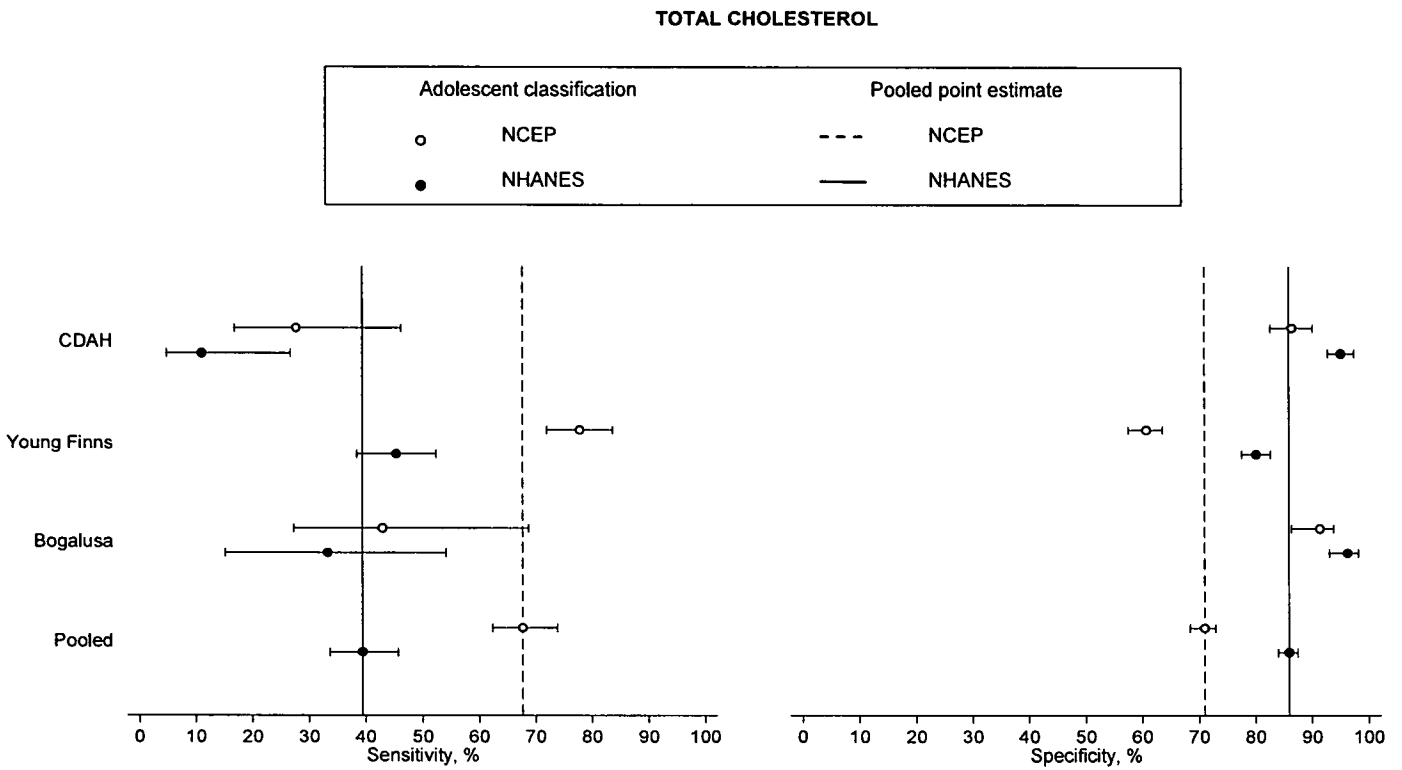


**Figure 31.** Sensitivity, specificity and 95% confidence intervals for predicting abnormal total cholesterol levels in adulthood for adolescents classified as high-risk using NCEP and NHANES cut-points.

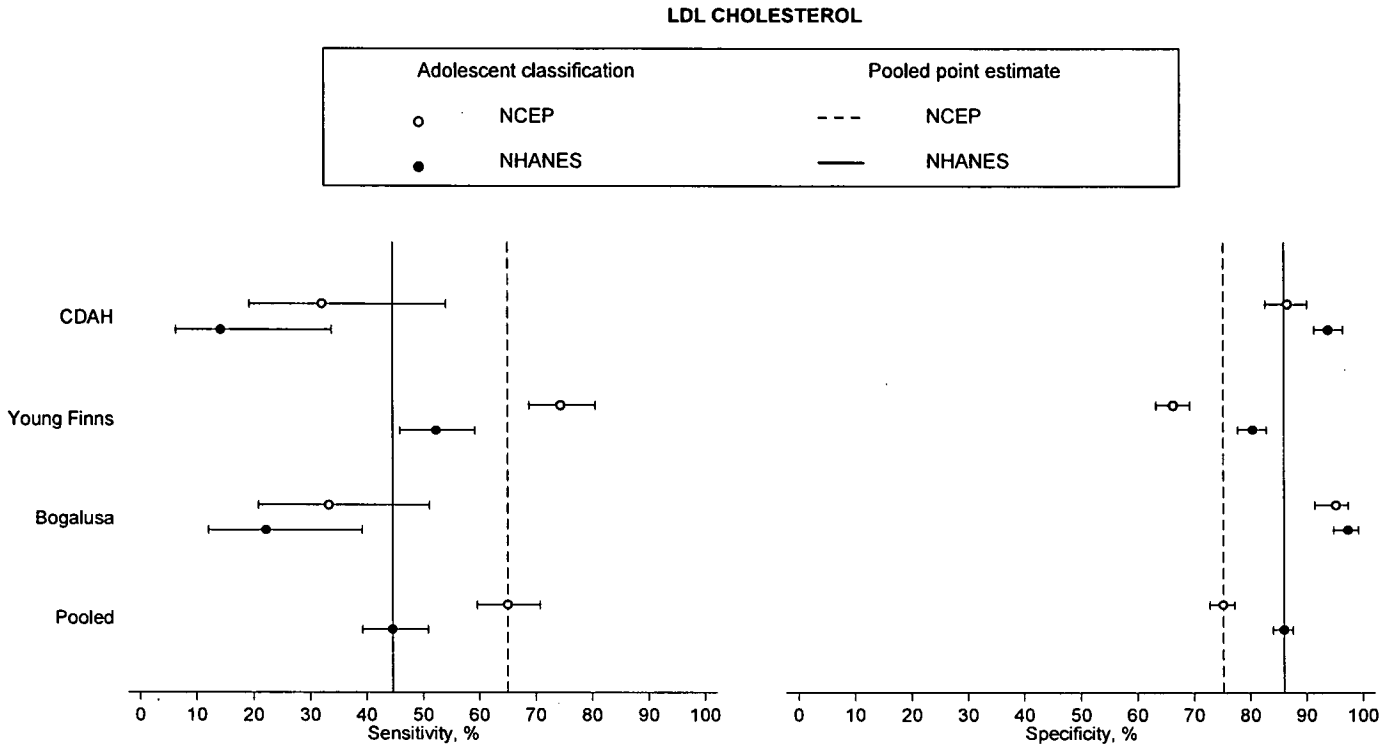


**Figure 32.** Sensitivity, specificity and 95% confidence intervals for predicting abnormal LDL cholesterol levels in adulthood for adolescents classified as high-risk using NCEP and NHANES cut-points.

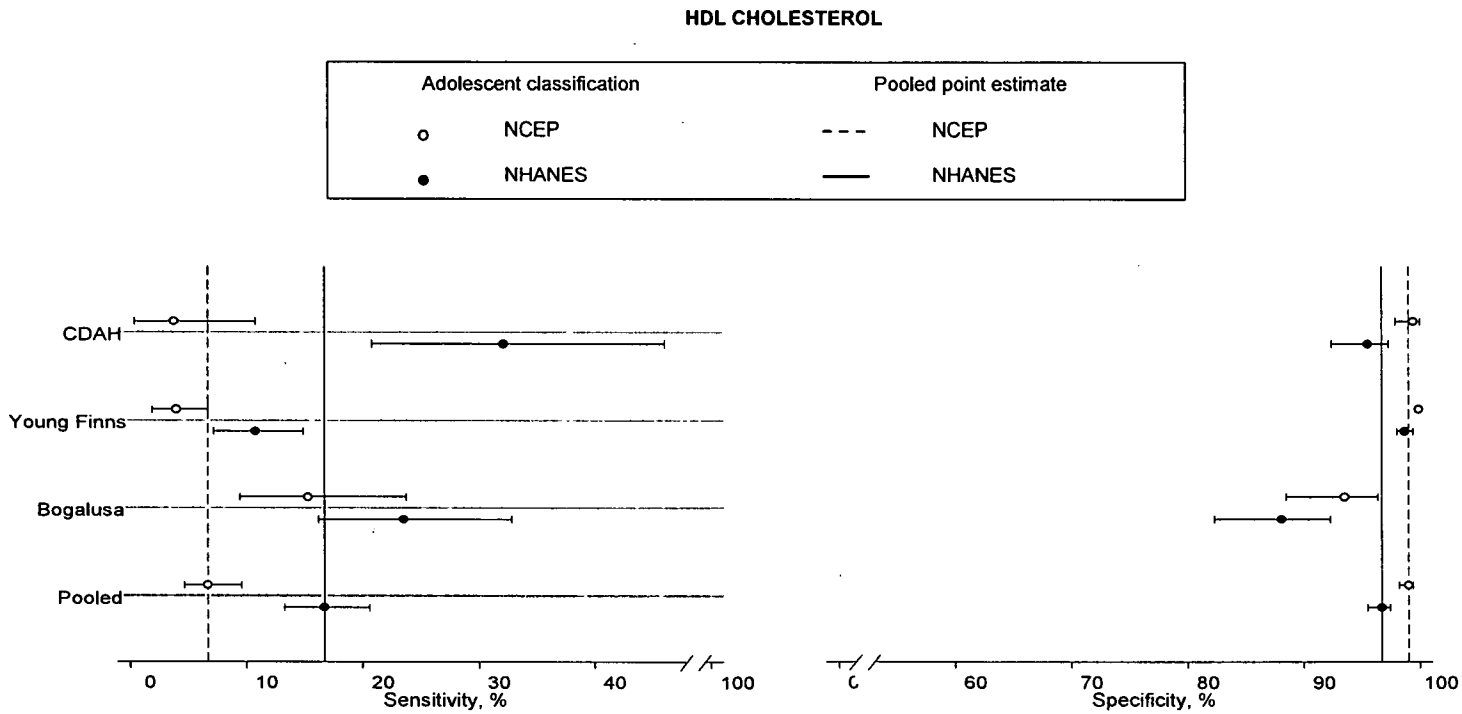


**Figure 33.** Sensitivity, specificity and 95% confidence intervals for predicting abnormal HDL cholesterol levels in adulthood for adolescents classified as high-risk using NCEP and NHANES cut-points.

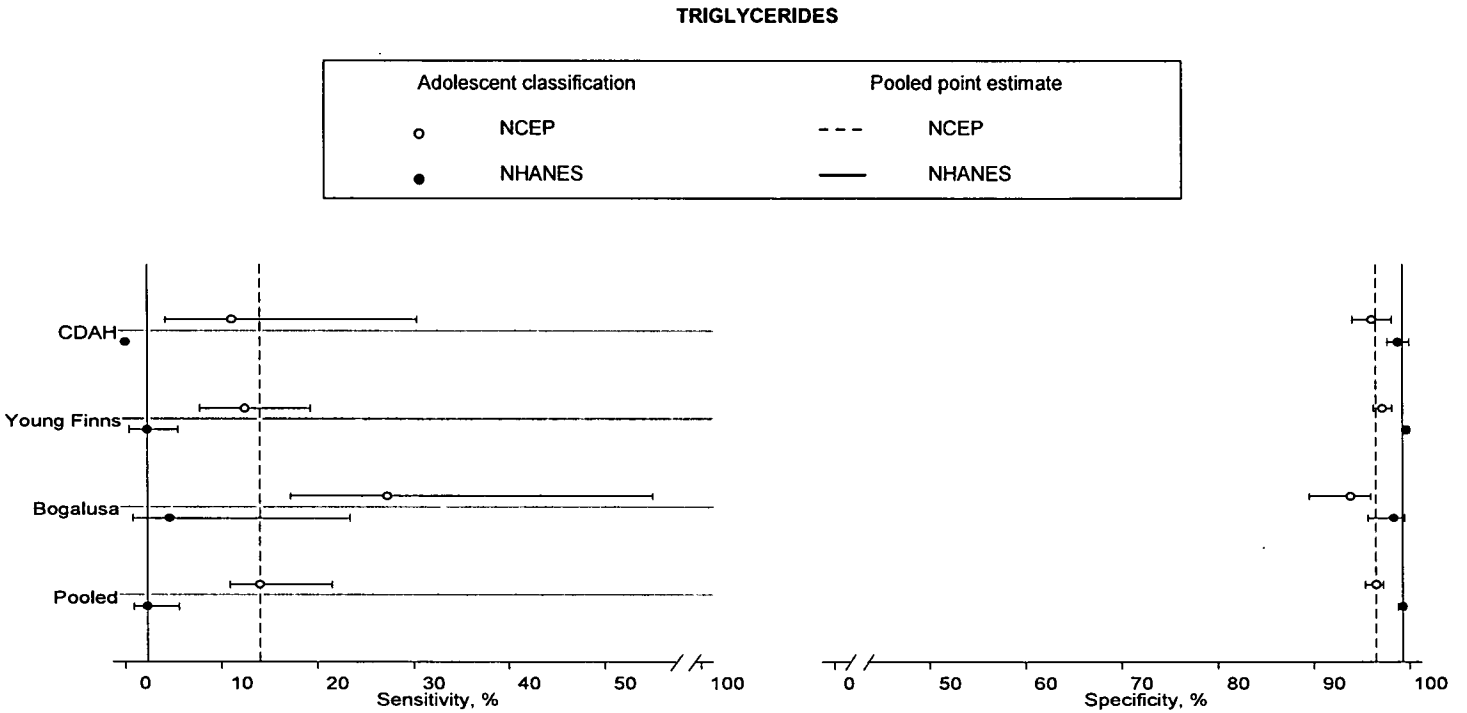


**Figure 34.** Sensitivity, specificity and 95% confidence intervals for predicting abnormal triglyceride levels in adulthood for adolescents classified as high-risk using NCEP and NHANES cut-points.

### 4.4.3 EVALUATION OF DIFFERENT SCREENING STRATEGIES

Sensitivity, specificity, PPV, NPV, and AUC for different screening strategies in the Young Finns cohort are presented in Table 20 using NCEP cut-points for total cholesterol, LDL cholesterol, and triglycerides and NHANES cut-points for HDL cholesterol. Additional illustrations and data comparing false positive and false negative rates (Figure 35), and the proportion identified/not identified and degree of overlap between screening strategies (Figure 36, Figure 37) are also presented. Because results for total cholesterol and LDL cholesterol, and results for HDL cholesterol and triglycerides were similar, the focus herein pertains to LDL cholesterol and HDL cholesterol only.

Adding positive family history and/or overweight-obesity status to the NCEP high-risk cut-point only marginally improved the identification of adolescent Young Finns who would develop abnormal LDL cholesterol levels as adults compared with universal screening. Forty-three participants (20%) with high LDL cholesterol levels at follow-up were not identified by any of the screening strategies (Figure 36). Universal screening identified 75% of participants with high LDL cholesterol levels at follow-up, but at a high trade-off in false positives (66.2%). The absolute numbers of participants identified using family history, overweight-obese, or family history and/or overweight-obese were considerably lower than universal screening, but false positive proportions remained high (58.5%, 66.7%, 62.6% respectively; Figure 35). Improvements in sensitivity, specificity, and PPV where observed when NHANES HDL cholesterol high-risk cut-points were combined with family history, overweight-obese status, or family history/overweight-obese compared with universal screening. Regardless of the adolescent screening strategy employed, most adults (71%) with low HDL cholesterol levels were not identified at baseline (Figure 37). Selective screening of HDL cholesterol in those with family history, overweight-obese, or family history/overweight-obese identified less total cases but at a considerably lower proportion of false positives compared with universal screening (Figure 35). Interestingly, screening adolescents using positive family history or overweight-obese status, without HDL cholesterol analysis, independently identified more total and unique at-risk individuals compared with universal HDL cholesterol screening (Figure 37).

**Table 20.** Capacity of different screening strategies to identify adolescent Young Finns who developed dyslipidemia in adulthood

| Lipoprotein variable | Screening strategy | N* | Sensitivity | Specificity | PPV | NPV | AUC |
|---|---|---|---|---|---|---|---|
| Total cholesterol | | | | | | | |
| | Universal | 1180 | 77.8 | 60.7 | 28.4 | 93.1 | 0.70 |
| | Positive family history† + cut-points | 174 | 80.5 | 60.2 | 38.4 | 90.9 | 0.71 |
| | Overweight‡ + cut-points | 87 | 100.0 | 58.3 | 33.3 | 100.0 | 0.79 |
| | Positive family history† OR Overweight‡ + cut-points | 241 | 84.0 | 58.6 | 34.7 | 93.3 | 0.72 |
| LDL cholesterol | | | | | | | |
| | Universal | 1158 | 74.4 | 66.4 | 33.8 | 91.9 | 0.73 |
| | Positive family history† + cut-points | 173 | 77.3 | 62.8 | 41.5 | 89.0 | 0.73 |
| | Overweight‡ + cut-points | 85 | 78.9 | 54.6 | 33.3 | 90.0 | 0.67 |
| | Positive family history† OR Overweight‡ + cut-points | 239 | 76.8 | 60.7 | 37.4 | 89.5 | 0.72 |
| HDL cholesterol | | | | | | | |
| | Universal | 1179 | 10.8 | 98.7 | 69.2 | 80.4 | 0.72 |
| | Positive family history† + cut-points | 174 | 15.6 | 99.3 | 83.3 | 83.9 | 0.72 |
| | Overweight‡ + cut-points | 87 | 29.0 | 100.0 | 100.0 | 71.4 | 0.71 |
| | Positive family history† OR Overweight‡ + cut-points | 241 | 20.7 | 99.5 | 92.3 | 79.8 | 0.73 |
| Triglycerides | | | | | | | |
| | Universal | 1180 | 12.5 | 97.2 | 35.6 | 90.0 | 0.61 |
| | Positive family history† + cut-points | 174 | 19.1 | 94.1 | 30.8 | 89.4 | 0.64 |
| | Overweight‡ + cut-points | 87 | 29.2 | 90.5 | 53.8 | 76.7 | 0.65 |
| | Positive family history† OR Overweight‡ + cut-points | 241 | 23.1 | 93.6 | 40.9 | 86.3 | 0.66 |

* Refers to the total number of participants that would be screened; † family history was considered positive if a participant's father *or* mother had been diagnosed with CHD, experienced myocardial infarction, or had percutaneous coronary intervention or coronary bypass surgery at ≤55 years of age at follow-up (2001); ‡ overweight or obese according to International Obesity Task Force BMI cut-points. Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the receiver-operating characteristic curve.

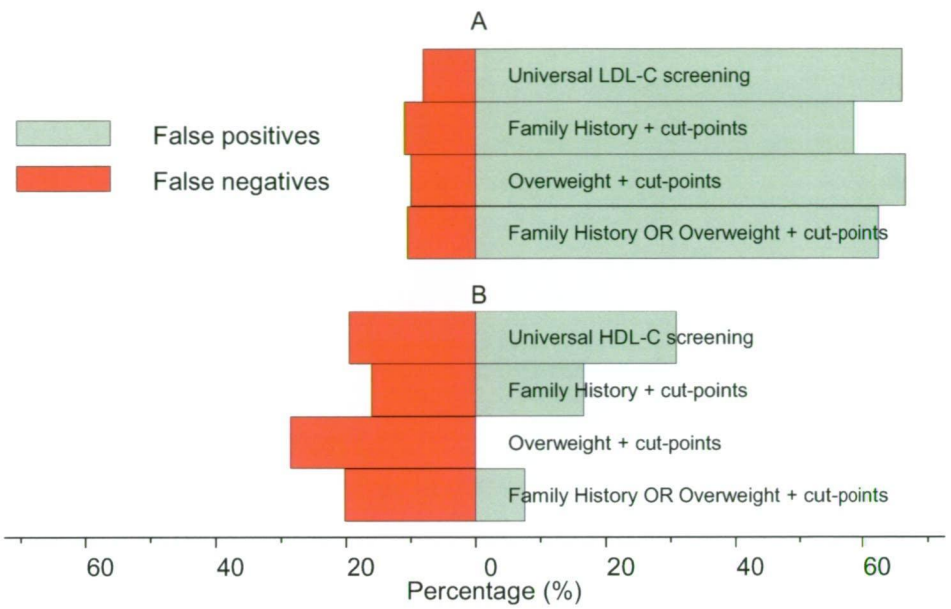


**Figure 35.** Proportions of adolescents identified for treatment that did not maintain high-risk levels at follow-up (false positives) and adolescents not identified for treatment that developed high-risk levels at follow-up (false negatives) according to different screening strategies for A) LDL cholesterol and B) HDL cholesterol levels in the Young Finns cohort.

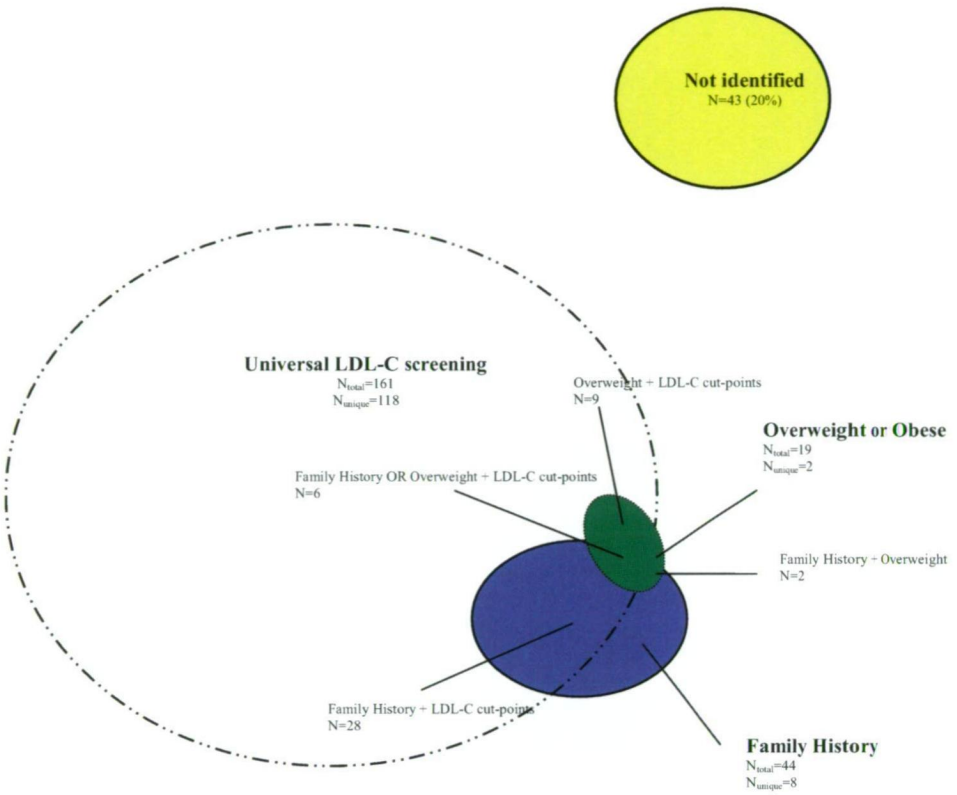


**Figure 36.** The number of Young Finns participants with high LDL cholesterol levels as adults (N=216) identified and not identified using different adolescent screening strategies. Abbreviations: $N_{total}$, the total number of participants identified in adolescence by separate screening strategies who developed high LDL cholesterol levels as adults; $N_{unique}$, the number of participants identified by unique combinations of screening criteria. The size of each ellipse is based on the proportion of total adult cases identified by childhood screening and is approximately to scale.

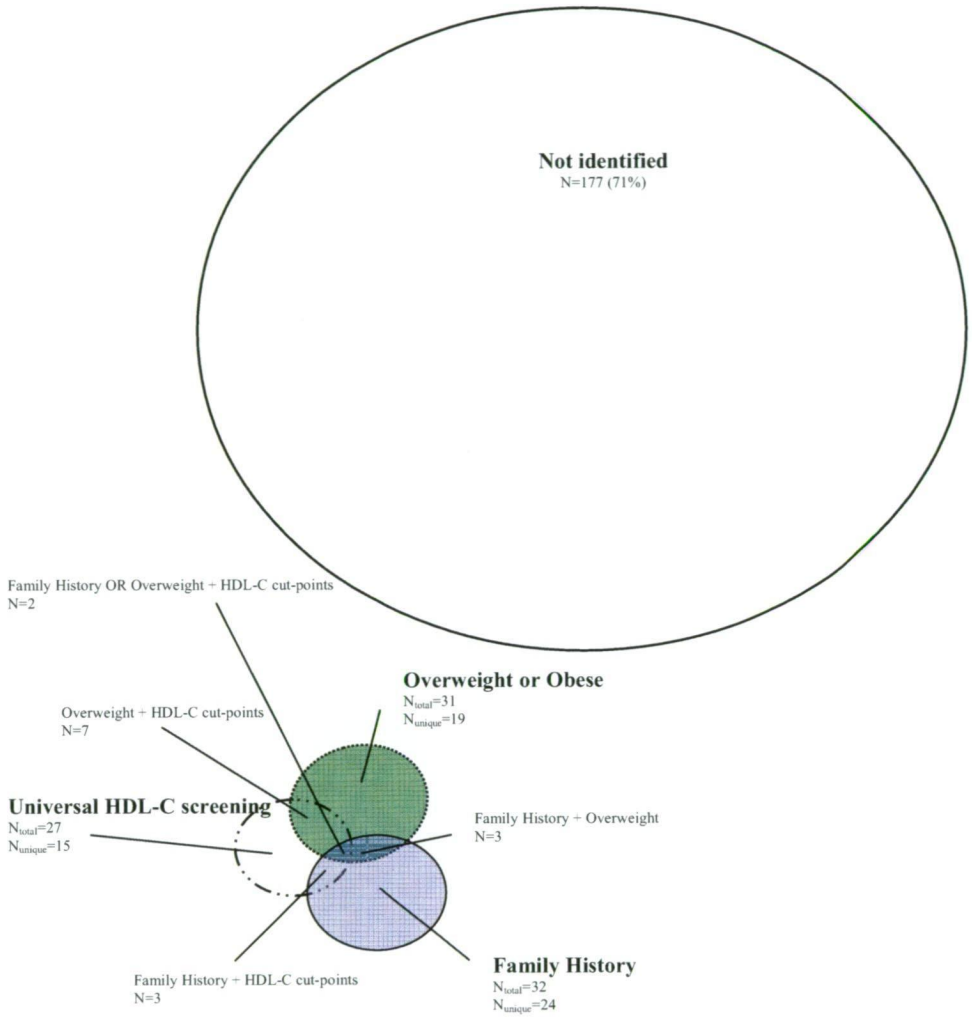


**Figure 37**. The number of Young Finns participants with low HDL cholesterol levels as adults (N=250) identified and not identified using different adolescent screening strategies. $N_{total}$, the total number of participants identified in adolescence by separate screening strategies who developed high LDL cholesterol levels as adults; $N_{unique}$, the number of participants identified by unique combinations of screening criteria. The size of each ellipse is based on the proportion of total adult cases identified by childhood screening and is approximately to scale.

## 4.5. DISCUSSION

Age- and sex-specific lipid and lipoprotein cut-points developed from NHANES data have been proposed to provide a more accurate classification of adolescents with lipid disorders compared with the NCEP classification. However, there have been no studies to assess whether the NHANES cut-points improve the prediction of those individuals that will develop associated dyslipidemia in adulthood. In this study, data from 1809 participants in three prospective cohort studies who had lipoprotein variables collected during adolescence and again in adulthood with a mean follow-up of 20.2 years were examined. After adjusting for potential confounding variables, pooled and cohort-stratified data showed that there is a progressive and substantial increase in the relative risk of adolescents with borderline- or high-risk lipoprotein levels, whether defined according to NCEP or NHANES cut-points, to develop associated dyslipidemia 15 to 20 years later. These findings are consistent with reports that have shown lipoprotein levels track from adolescence to adulthood,[161, 162] and underline the usefulness of evaluating lipoprotein variables to identify adolescents who may benefit from intervention. The present study directly compared differences in the predictive capacity of the NCEP and NHANES lipoprotein variable cut-points. The recently proposed NHANES cut-points for HDL cholesterol offered a better prediction of those adolescents most likely to develop abnormal levels in adulthood, while the predictions for total cholesterol, LDL cholesterol, and triglycerides were poorer than those achieved with the NCEP classification. It was evident though, that neither classification proved clearly better for prediction. These results are surprising given that the new NHANES cut-points recognise age and sex shifts during adolescence, and are linked to evidence- and health-based adult NCEP ATPIII thresholds. In interpreting these data, it is necessary to take into account the populations examined in this study, as well as consider the practical implementation of these findings to revision of paediatric cut-points in a likely update to the guidelines by the NHLBI Pediatric Cardiovascular Risk Reduction Initiative.

Because the cohorts used in this study collected baseline data in the early to mid 1980s, it is necessary to consider secular trends in lipid and lipoprotein data over this time in consideration of the performance of both NCEP and NHANES cut-points. Secular trend data from the Young Finns cohort have showed modest decreases in total cholesterol, and LDL cholesterol, with more substantial decreases for HDL cholesterol, and increases in triglyceride levels in both adolescents[217] and young adults[218] since 1980. Moreover, when the source populations for each adolescent classifications are examined, it is evident that levels

of total cholesterol, LDL cholesterol, HDL cholesterol were lower and triglycerides levels higher in the NHANES studies by comparison with the LRC Prevalence Study,[196] from which the NCEP cut-points were derived. This trend is consistent when adolescent lipid and lipoprotein variable levels in CDAH,[365] Young Finns,[366] and Bogalusa cohorts are compared with levels from NHANES. This is not surprising given that baseline data were collected prior to the more recent obesity epidemic; whereas the NHANES data were collected during this period. It is possible then, that the observed gains in predictive power of the NCEP cut-points in these data could be attributed to the period when the cut-points were developed (reflecting the population distribution at the time). It can not be discounted that the NHANES cut-points may provide a more accurate classification of today's adolescents. Prospective studies of the type used in this study, with periods of follow-up that span adolescence and early adulthood, will always be subject to this limitation.

Heterogeneity between cohort data was apparent. Although comparisons of NCEP vs. NHANES cut-points showed similar patterns within cohorts, the relative value of the cut-points in each population differed considerably. Discrepancies in the sensitivity and specificity of high-risk cut-points were evident between studies when each classification was considered separately (Figure 2), particularly for total cholesterol and LDL cholesterol. For example, in adults with abnormal total cholesterol levels, 27.8 % of Australian, 77.8 % of Finn, and 42.9 % of US participants would have been identified in childhood using the NCEP high-risk cut-point. The value of these cut-points for screening should be considered in different population settings. For example, the efficacy of universal screening using the NCEP total cholesterol cut-points in a Finnish population would be different from that in an Australian population. Selection bias is unlikely to explain these differences, since baseline lipoprotein variables for participants and non-participants at follow-up were similar in each cohort.

Noticeable differences in lipid and lipoprotein levels were apparent between countries in these data, which may partly explain the degree of heterogeneity observed. Because different methods were used for lipid and lipoprotein determination it would be erroneous to conclude that these necessarily reflect population differences. For example, several studies have shown that HDL cholesterol levels vary depending on the method of determination.[367-369] Using standardised methods for lipid determination, Knuiman et al demonstrated between country differences in total cholesterol and HDL cholesterol concentrations in 7 to 8 year old boys from 16 countries;[211] and differences in HDL

cholesterol concentrations in 33 to 48 year old males from 13 countries.[370] The heterogeneity observed in this study is likely a function of both actual differences and methodological differences. It is reassuring that the best performing classifications for each lipoprotein variable were consistent between cohorts despite differences in laboratory methodology for lipoprotein variable determination.

The existing paediatric guidelines recommend targeted lipoprotein screening in children and adolescents with a positive family history of premature CHD *or* high total cholesterol levels (≥6.2 mmol/L; 240 mg/dL), with a recent update recommending screening children who are overweight or obese.[220, 249] The screening data in the Young Finns cohort did not provide a clear indication on the best screening approach (universal vs. selective) for use in adolescents. For example, universal screening and selective screening for total cholesterol and LDL cholesterol are not acceptable with rates of false positives that suggest approximately 60% of adolescents identified as having high-risk levels would not have abnormal levels in early adulthood, which is strikingly consistent with the study from Friedman et al. that showed false positive rates of 61% and 69% respectively in data from the 25 year follow-up of the Princeton LRC Prevalence Program.[276] However, universal screening comes with the benefit of identifying 75% of those affected in adulthood. Neither universal nor selective screening for HDL cholesterol or triglycerides was efficient in identifying those adolescents that developed abnormal levels as adults. Although selective HDL cholesterol screening reduced the false positive rate compared with universal screening, the strategies were inefficient at identifying adolescents who developed low HDL cholesterol levels as adults. This is highlighted by the fact that using overweight or obesity status, or positive family history alone to indicate an adolescent's risk identified more total cases than any form of HDL cholesterol screening. In consideration of these findings with current paediatric recommendations that endorse a selective screening approach, clinicians need to observe that a substantial number of adolescents with LDL cholesterol abnormalities may not have high-risk levels in early adulthood, and that most individuals that develop low HDL cholesterol levels as adults may not be identified in adolescence.

### 4.5.1 LIMITATIONS

Several limitations were evident. First, due to the recognised within-person variability of lipid and lipoprotein levels,[308-310] misclassification of lipoprotein status may have occurred by using a single lipoprotein measurement at baseline and follow-up. In sensitivity analyses, the

possible effect of misclassification was examined by comparing AUC of single and repeated adolescent measurements for Young Finns who had data collected in 1980 and 1983 and who remained adolescents. Consistent with the literature,[337] a single lipid or lipoprotein measurement underestimated the strength of association with the outcome when compared with repeated measures. Increases in AUC were in the order of 3 to 7% for total cholesterol, LDL cholesterol and HDL cholesterol, but was considerably higher for repeated triglyceride measurements (7% and 13% for NHANES and NCEP respectively) (see Appendix 9 for complete data). While these findings, based on a single lipid or lipoprotein measurement in adolescence, likely underestimate the ability of both paediatric classifications to predict associated adult dyslipidemia, it was reassuring that the best performing classification for each lipid and lipoprotein variable was consistent with the primary analyses and conclusions presented. Second, data from the Bogalusa Heart Study have not only shown differences in the distribution of lipid and lipoprotein levels by race, but that dynamic changes in blood lipid and lipoprotein levels that occur at the onset of puberty are differentially influenced by race. For example, HDL cholesterol levels decline rapidly in Caucasian boys at the onset of puberty, whereas LDL cholesterol levels fall in African-American boys.[209, 300] Consequently, owing to the low number of African Americans in this study, these findings should be applied with caution to bi-racial populations until more data are available. Third, this study used lipid and lipoprotein risk status in adulthood as the outcome of interest and did not establish whether the two adolescent classifications predict clinically relevant end-points such as the presence and progression of atherosclerosis, or CVD events. Fourth, cut-points derived from, and intended for, US populations to Australian and Finnish data were used. Although these data suggest the adolescent cut-points could be generalised to other populations of European ancestry, applying them without consideration of possible country or region specific differences may not be optimal. Finally, the measure of family history of premature CHD in the Young Finns cohort was acquired at follow-up from self-report, and not during baseline examination or from hospital records or death certificates.[371] It is likely that the definition used here (pertaining to parental history) would have indicated several less children if it were collected in childhood. Moreover, research examining the net bias in both selection and recall for self-report measures of family history has shown a shifting of the true effect toward the null,[371, 372] suggesting the estimates of prediction for family history in this study were likely conservative.

## 4.6. CONCLUSIONS

Although neither classification proved clearly better for prediction of dyslipidemia in adulthood, the data suggest that the separate use of NHANES cut-points for HDL cholesterol and NCEP cut-points for total cholesterol, LDL cholesterol, and triglycerides may provide the most accurate classification of what constitutes normal-, borderline-, and high-risk lipid and lipoprotein levels in adolescents. In addition, the data highlighted substantial limitations, from an epidemiological perspective, to universal and targeted screening approaches to identifying youth with high-risk blood lipid and lipoprotein levels in young adulthood.

**KEY POINTS**

- Two groups (the National Cholesterol Education Program, NCEP;[2] and Jolliffe & Janssen,[3] NHANES) have circulated paediatric cut-points of what constitutes normal-, borderline-, and high-risk lipoprotein variable levels.

- No study has assessed which of these classifications are most effective at predicting those adolescents who will develop abnormal levels in adulthood.

- Using pooled data from three prospective cohort studies from Australia, Finland, and the United States, the results suggest that clinicians wishing to identify lipid disorders in adolescents may improve risk stratification by the separate adoption of cut-points for HDL cholesterol stipulated by Jolliffe and Janssen, and NCEP stipulated cut-points for total cholesterol, LDL cholesterol, and triglycerides.

- The data also suggest that clinicians employing current paediatric guidelines for targeted lipoprotein screening in children and adolescents with a positive family history of premature CHD or who are overweight or obese, need to consider that a substantial number of those adolescents identified with high total cholesterol or LDL cholesterol levels may not have high-risk levels in early adulthood, and that most individuals that develop abnormal HDL cholesterol or triglyceride levels as adults may not be identified in adolescence.

**Box 4.** Summary of key points from Chapter 4: utility of two currently recommended North American paediatric dyslipidemia classifications to predict dyslipidemia in adulthood

# 5. THE ASSOCIATION OF PAEDIATRIC LOW- AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL DYSLIPIDEMIA CLASSIFICATIONS AND CHANGE IN DYSLIPIDEMIA STATUS WITH CAROTID INTIMA-MEDIA THICKNESS IN ADULTHOOD

## 5.1. INTRODUCTION

The previous chapter evaluated the ability of two classifications of paediatric dyslipidemia to predict dyslipidemia in adulthood using data from three prospective cohort studies. Both borderline- and high-risk cut-points stipulated by NCEP,[196] or age- and sex-specific cut-points based on distributions from NHANES proposed by Jolliffe and Janssen[251] were shown to predict a progressive and substantial increase in the relative risk of dyslipidemia in adulthood. Although the data suggested that prediction of adult dyslipidemia may be improved by the separate adoption of the NCEP cut-points for total cholesterol, LDL cholesterol, and triglycerides, and NHANES cut-points for HDL cholesterol, it was evident that neither classification proved substantially better. The data also suggested that while adolescent dyslipidemia status predicted adult dyslipidemia, most of those with paediatric high-risk levels did not have high-risk levels as adults, and, that the use of multiple potential screening strategies did not substantially improve this statistic. While these data are important, they do not provide evidence of the utility of paediatric dyslipidemia classifications to predict CVD or markers of atherosclerosis. While each of these (still young) cohorts will have to wait for clinical events to amass to a point that analyses using clinical outcomes can be performed, non-invasive assessments of preclinical markers of atherosclerosis (such as carotid intima-media thickness, IMT) that reflect change in the vasculature provide useful intermediary phenotypes of the disease process. That is, they allow the assessment of early atherosclerosis long before clinical manifestations become apparent.

As outlined briefly in section 1.3, the ultrasonic assessment of carotid IMT provides a non-invasive, safe, quick, relatively inexpensive, and reproducible measurement of vascular structure that correlates with CVD risk factors,[373] coronary artery disease,[374] and is a predictor of future vascular events.[375] An overview of population-based cohort studies that have

examined the ability of carotid intima-media thickness to predict clinical cardiovascular events is presented in Appendix 10. Because of these features, carotid IMT is suitable for large epidemiological studies. As outlined in the Methods chapter, each of the three cohorts (CDAH, Young Finns, and Bogalusa) used in this study conducted B-mode ultrasound scans of the carotid artery. A useful addition to the data presented in the previous chapter would therefore be to examine the utility of the paediatric cut-points to predict carotid IMT.

## 5.2. AIMS

The aims were:

1. To determine which of the NCEP or NHANES adolescent LDL cholesterol and HDL cholesterol dyslipidemia classifications best predict high common carotid IMT in adulthood; and
2. To assess whether maintaining or changing dyslipidemia status from adolescence to adulthood has an effect on carotid IMT measured in adulthood.

## 5.3. METHODS

To address the aims, data from the three population-based, prospective cohort studies outlined in chapter 2 were used.

### Australian Data: The Childhood Determinants of Adult Health (CDAH) Study

*Study sample*

The CDAH sample has been described in section 2.2. For these analyses, data from 286 participants (26% of those eligible from baseline, 50% male) aged 12 and 15 years at baseline who had LDL cholesterol and HDL cholesterol data from 1985 and carotid artery ultrasound measures at follow-up (2004-2006) were included.

*Clinic measurements*

Baseline and follow-up measurements of blood lipid and lipoproteins, blood pressure, BMI, and smoking habits were collected according to protocols described in section 2.2.3.

*Carotid artery ultrasound studies*

B-mode ultrasound studies of the left carotid artery were performed according to the protocols outlined in section 2.2.3.5.

### Finnish Data: The Cardiovascular Risk in Young Finns Study

*Study sample*

The Young Finns sample is described in section 2.3. For these analyses, 1171 subjects (66% of those eligible, 45 %male) who were aged 12, 15, and 18 years at baseline and who had LDL cholesterol and HDL cholesterol data from 1980 and carotid artery ultrasonography data in 2001 were included.

*Clinic measurements*

Protocols for baseline and follow-up measures of blood lipids and lipoproteins, blood pressure, BMI, and cigarette smoking have been described in section 2.3.3.

*Carotid artery ultrasound studies*

B-mode ultrasound studies of the left carotid artery were performed at follow-up using standardised protocols that were described in section 2.3.3.5.

**United States Data: The Bogalusa Heart Study**

*Study sample*

The Bogalusa Heart study sample has been described in section 2.4. For these analyses, 254 participants (17% of those eligible from the 1984-1985 survey, 44% male, 30% African American) aged 12 to 17 years at baseline who had LDL cholesterol and HDL cholesterol measures from baseline (1984-1985) and carotid artery ultrasound at follow-up (2001-2002) were included.

*Clinic measurements*

Baseline and follow-up measures of blood lipid and lipoproteins, blood pressure, BMI, and cigarette smoking have been described in section 2.4.3.

*Carotid artery ultrasound studies*

B-mode ultrasound examinations of the left and right carotid artery were performed according to protocols described in section 2.4.3.5.

### 5.3.1 CLASSIFICATION OF LDL CHOLESTEROL STATUS IN ADOLESCENCE AND ADULTHOOD

NCEP[196] and NHANES[251] paediatric cut-points were used to assign adolescent LDL cholesterol and HDL cholesterol status (see Table 13, chapter 4). At follow-up, NCEP adult treatment panel guidelines were used to classify participants as non-dyslipidemic (<4.14 mmol/L; <160 mg/dL), or dyslipidemic (≥4.14 mmol/L; ≥160 mg/dL) LDL cholesterol status, and non-dyslipidemic (≥1.036 mmol/L; ≥40 mg/dL) or dyslipidemic (<1.036 mmol/L; <40 mg/dL) HDL cholesterol status.[304]

### 5.3.2 CLASSIFICATION OF BLOOD PRESSURE STATUS IN ADOLESCENCE AND ADULTHOOD

Participants aged ≤ 17 years of age at baseline were classified according to age, sex, and height percentiles for blood pressure issued by the National High Blood Pressure Education Program.[305] Blood pressure status in adolescence was classified as normal if systolic and diastolic blood pressures were <90$^{th}$ percentile, prehypertensive if systolic or diastolic blood

pressure were ≥90$^{th}$, but <95$^{th}$ percentile, and hypertensive if systolic or diastolic blood pressure was ≥95$^{th}$ percentile. For participants aged 18 years at baseline, blood pressure was classified as normal if systolic was <120 mm/Hg and diastolic <90 mm/Hg, prehypertensive if systolic was 120-139 mm/Hg or diastolic blood pressure was 80-89 mm/Hg, and hypertensive if systolic ≥140 mm/Hg or diastolic blood pressure was ≥90 mm/Hg according to the Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[376]

### 5.3.3 CLASSIFICATION OF OVERWEIGHT AND OBESITY STATUS IN ADOLESCENCE AND ADULTHOOD

Adolescents aged ≤ 17 years of age at baseline were classified as normal weight, overweight, or obese according to age- and sex-specific BMI cut-points endorsed by the International Obesity Task Force.[306] Participants aged 18 years at baseline were classified as normal weight (BMI <25kg/m$^2$), overweight (BMI ≥25 to <30kg/m$^2$), or obese (BMI ≥30kg/m$^2$) as per classifications outlined by the WHO.[280]

### 5.3.4 CAROTID IMT MEASUREMENTS AND CLASSIFICATION OF HIGH CAROTID IMT IN ADULTHOOD

For the analyses, the most consistent IMT measurement across the three study sites was selected. This incorporated the maximum measurement at the far wall of the left common carotid artery (see Table 21 and Figure 38). While no consensus clinical definition of high carotid IMT currently exist for young adults, high IMT in adulthood was defined as a maximum IMT ≥90$^{th}$ percentile for age, sex, race (Bogalusa), and cohort specific values. The specific values used to denote high IMT are provided in Table 22.

**Table 21.** Comparison of common carotid IMT definitions obtained in Young Finns, Bogalusa, and CDAH populations.

| Cohort | N | Mean age at baseline, y | Mean length of follow-up, y | IMT definition | | | | Mean maximal IMT, mm | Average absolute difference of replicate measures ± SD, mm |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Carotid artery | Wall | Segment/s | Measurements | | |
| Young Finns | 1171 | 14.9 | 21.0 | Left | Far | CCA | Mean & Max IMT | 0.65±0.10 | 0.05±0.04 |
| Bogalusa | 254 | 15.3 | 17.1 | Left & right | Far | ICA, BIF, CCA | Max IMT | 0.73±0.14 | 0.05±0.03 |
| CDAH | 286 | 13.4 | 20.0 | Left | Far | CCA | Mean & Max IMT | 0.62±0.10 | 0.02±0.04 |

Abbreviations: CCA, common carotid artery; BIF, carotid bifurcation; ICA, internal carotid artery.

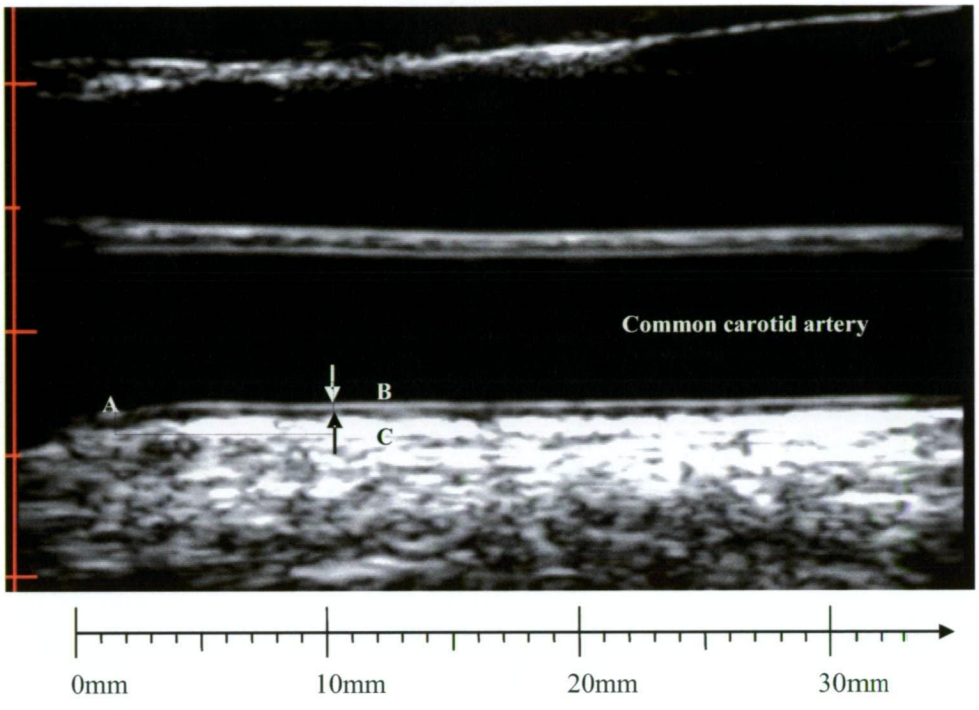


**Figure 38.** B-mode ultrasound image of the left common carotid artery: A) border of carotid bulb widening (0 mm), B) common carotid artery far wall lumen-intima interface, C) media-adventitia interface. Definitions of the common carotid artery segment in each cohort: Young Finns and CDAH, common carotid artery IMT is taken as the distance between B and C measured in the vicinity 10 mm proximal to the border of the bulb widening (A); Bogalusa, common carotid artery IMT is taken as the distance between B and C measured 0-10 mm proximal to the border of the bulb widening (measurement area highlighted in yellow).

**Table 22.** Age, sex, race (Bogalusa only), and cohort specific 90th percentile cut-points of maximum common carotid IMT (mm) used in each study to denote high IMT

| | Young Finns | | Bogalusa | | | | CDAH | |
|---|---|---|---|---|---|---|---|---|
| | | | Caucasian | | African Americans | | | |
| Age | M | F | M | F | M | F | M | F |
| 29 | - | - | 0.925 | 0.805 | 0.983 | 0.898 | 0.715 | 0.660 |
| 30 | - | - | 0.934 | 0.813 | 0.993 | 0.908 | 0.776 | 0.704 |
| 31 | - | - | 0.942 | 0.821 | 0.993 | 0.908 | 0.770 | 0.715 |
| 32 | - | - | 0.951 | 0.829 | 1.013 | 0.929 | 0.770 | 0.682 |
| 33 | 0.770 | 0.720 | 0.960 | 0.837 | 1.023 | 0.940 | 0.770 | 0.715 |
| 34 | - | - | 0.969 | 0.845 | 1.033 | 0.950 | 0.825 | 0.748 |
| 35 | - | - | 0.978 | 0.853 | 1.043 | 0.960 | 0.770 | 0.693 |
| 36 | 0.790 | 0.770 | - | - | - | - | - | - |
| 39 | 0.864 | 0.770 | - | - | - | - | - | - |

M = males; F = females

## 5.3.5 STATISTICAL ANALYSES

Comparisons between baseline characteristics of participants and non-participants at follow-up within each cohort were performed using logistic regression. Participation (yes/no) was used as the binary dependent variable in these analyses.

*Comparison of NCEP vs. NHANES adolescent dyslipidemia classifications*

Log-binomial regression was used to estimate the relative risk of high IMT at follow-up, for various baseline lipoprotein classifications.[377] Estimates were adjusted for age at baseline, sex, race (for the Bogalusa data only), and length of follow-up. The adjustment for length of follow-up was to account for within-cohort differences observed between length of follow-up and risk of high IMT in both the CDAH and Bogalusa studies. For pooled estimates, a term for cohort was included in the models to account for differences in lipoprotein determination methods between the three cohorts. Baseline BMI status (normal weight vs. overweight/obese in adolescence)[306] was also considered as a potential confounding variable in these analyses. The analyses for NCEP and NHANES HDL cholesterol classifications were stratified by BMI status due to effect modification.

The ability of each adolescent LDL cholesterol and HDL cholesterol classification to predict high carotid IMT in adulthood was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under receiver-operating characteristic curves (AUC). Tests for significant differences of sensitivity and specificity between NCEP and NHANES adolescent cut-points were performed using McNemar's test.[363] Confidence intervals for sensitivity and specificity were calculated using the binomial distribution. Tests for significant differences between AUC were calculated using the DeLong algorithm.[364] This method assumes the correct null distribution when there are only three classification levels (i.e. normal-, borderline, and high-risk groups), as is the case here. Analyses for HDL cholesterol classifications were stratified by BMI status owing to effect modification.

*Change in dyslipidemia status and high IMT in adulthood*

A change variable was created that divided participants into four categories depending on their lipoprotein status at both time-points. Participants were classified as *persistent non-dyslipidemia, non-dyslipidemia to dyslipidemia, dyslipidemia to non-dyslipidemia* and *persistent dyslipidemia*. Their adolescent lipoprotein status was determined using what was

judged to be the best performing LDL cholesterol and HDL cholesterol paediatric dyslipidemia classifications for predicting adult IMT, ascertained from the above mentioned comparison analyses. Log binomial regression was used to estimate the relative risk of high carotid IMT in adulthood depending on the change variable. These analyses were performed for pooled data only and are presented adjusted for age at baseline, sex, cohort and length of follow-up in model 1, and with additional adjustments for BMI at baseline, systolic blood pressure at baseline, and smoking status at baseline in model 2. Interaction between the model variables was tested; no significant interactions were found.

To compliment the analysis of categorical variables, the effect of change in the continuous lipoprotein levels on the continuous outcome measure of IMT using linear regression was also examined. The life-course analysis approach described by De Stavola et al. was adopted,[378] with the adolescent lipoprotein variable and change in the lipoprotein variable (derived as the difference between adult and adolescent lipoprotein levels) used as predictor variables. The regression coefficient for the adolescent lipoprotein variable quantifies the total effect, i.e. the sum of direct and indirect (mediated via adult lipoprotein levels due to tracking)[161, 162] effects of adolescent LDL cholesterol or HDL cholesterol on IMT, whereas the regression coefficient for change in lipoprotein levels quantifies the additional gain from increasing or decreasing lipoprotein levels by 1 mmol/L between adolescence and adulthood relative to participants with the same adolescent lipoprotein level, but whose lipoprotein level was unchanged. Multivariable models were specified, including adolescent LDL cholesterol and change in LDL cholesterol; and adolescent HDL cholesterol and change in HDL cholesterol. All models were adjusted for age at baseline, sex, cohort, length of follow-up, baseline BMI, baseline systolic blood pressure, and baseline smoking status. The assumption of linearity in the regression models of the continuous predictor variables was checked using the method of fractional polynomials.[379] The analyses are presented stratified by adolescent BMI status due to effect modification. The distribution of IMT values were somewhat right-skewed, however for ease of interpretation and comparability with previous studies in young adults, non-transformed IMT values were used. Using log-transformed IMT values made little difference to the results presented.

## 5.4. RESULTS

### 5.4.1 CHARACTERISTICS OF PARTICIPANTS AND NON-PARTICIPANTS

Non-participation in those eligible for follow-up measures (34% Young Finns, 82% Bogalusa, 67% CDAH) was more likely in males and those of younger age in each cohort, and more likely in African-Americans in the Bogalusa cohort (Table 23). There were no statistically or clinically significant differences in LDL cholesterol or HDL cholesterol levels, or LDL cholesterol or HDL cholesterol dyslipidemia status between participants and non-participants in any of the three cohorts (Table 23).

**Table 23.** Baseline characteristics of participants and non-participants in follow-up surveys of each study.

| | | Young Finns | | Bogalusa | | CDAH | |
|---|---|---|---|---|---|---|---|
| | | Participants | Non-participants | Participants | Non-participants | Participants | Non-participants |
| N | | 1171 | 615 | 254 | 1256 | 286 | 817 |
| Age at baseline, y* | | 14.9±2.4 | 14.7±2.4 | 14.8±1.5 | 14.1±1.6* | 13.4±1.5 | 13.4±1.5 |
| Males, %† | | 44.7 | 55.5* | 43.7 | 52.0* | 50.0 | 55.3 |
| African American, %† | | - | - | 29.9 | 36.5* | - | - |
| LDL cholesterol, mmol/L | | 3.30±0.79 | 3.31±0.86 | 2.47±0.66 | 2.42±0.58 | 2.69±0.69 | 2.67±0.67 |
| HDL cholesterol, mmol/L | | 1.55±0.30 | 1.53±0.32 | 1.48±0.51 | 1.57±0.53 | 1.44±0.29 | 1.40±0.30 |
| NHANES LDL cholesterol, % | Normal | 13.2 | 15.0 | 52.6 | 53.9 | 38.5 | 37.9 |
| | Above-normal | 31.7 | 30.9 | 31.9 | 33.8 | 37.8 | 35.4 |
| | Borderline-high | 29.6 | 28.1 | 9.6 | 9.6 | 16.8 | 19.6 |
| | High | 25.5 | 26.0 | 6.0 | 2.7 | 7.0 | 7.1 |
| NCEP LDL cholesterol, % | Normal | 31.0 | 31.1 | 79.7 | 79.8 | 64.3 | 64.3 |
| | Borderline-high | 27.7 | 26.5 | 10.4 | 13.7 | 21.0 | 20.3 |
| | High | 41.3 | 42.4 | 10.0 | 6.6 | 14.7 | 15.3 |
| NHANES HDL cholesterol, % | Normal | 48.0 | 41.6 | 44.5 | 51.4 | 31.8 | 29.3 |
| | Borderline-low | 48.8 | 55.3 | 38.6 | 35.0 | 59.8 | 59.7 |
| | Low | 3.3 | 3.1 | 16.9 | 13.7 | 8.4 | 11.0 |
| NCEP HDL cholesterol, % | Normal | 45.4 | 42.3 | 38.6 | 49.6 | 31.8 | 27.3 |
| | Borderline-low | 53.6 | 56.1 | 50.4 | 41.1 | 66.8 | 69.7 |
| | Low | 0.9 | 1.6 | 11.0 | 9.3 | 1.4 | 3.1 |

Statistics are means ± SD for continuous variables and percentages for dichotomous variables. Differences between participants and non-participants examined using logistic regression. Analyses comparing differences in lipoprotein levels/proportions are adjusted for age and sex, with Bogalusa analyses additionally adjusted for race. *P<0.05

### 5.4.2 ADOLESCENT AND ADULT CHARACTERISTICS BY CAROTID IMT STATUS

Mean values for key baseline and follow-up variables are displayed in Table 24 for males and females in each cohort who had IMT $\geq 90^{th}$ percentile and IMT $<90^{th}$ percentile. Across each sex and cohort, those with high IMT at follow-up tended to have higher LDL cholesterol levels during adolescence and adulthood. Bogalusa participants and females in the CDAH study with high IMT had lower HDL cholesterol levels during adolescence but not at follow-up.

**Table 24.** Mean values for adolescent and adult characteristics in those with and without IMT ≥90th percentile in adulthood in cohorts from Finland, the United States, and Australia

| | | Males | | Females | | All |
|---|---|---|---|---|---|---|
| Cohort | | IMT <90th % | IMT ≥90th % | IMT <90th % | IMT ≥90th % | |
| Young Finns | N | 469 | 54 | 566 | 82 | 1171 |
| Adolescence | age, y | 15.0±2.4 | 14.8±2.4 | 14.8±2.4 | 15.0±2.5 | 14.9±2.4 |
| | LDL-C, mmol/L | 3.22±0.78 | 3.49±0.84 | 3.34±0.79 | 3.39±0.79 | 3.30±0.79 |
| | HDL-C, mmol/L | 1.50±0.31 | 1.49±0.33 | 1.59±0.29 | 1.57±0.29 | 1.55±0.30 |
| Adulthood | age, y | 36.0±2.4 | 35.8±2.4 | 35.8±2.4 | 36.0±2.5 | 35.9±2.4 |
| | LDL-C, mmol/L | 3.63±0.92 | 3.84±0.83 | 3.25±0.78 | 3.35±0.80 | 3.43±0.87 |
| | HDL-C, mmol/L | 1.18±0.28 | 1.15±0.31 | 1.40±0.30 | 1.37±0.31 | 1.30±0.31 |
| | IMT, mm | 0.64±0.08 | 0.87±0.11 | 0.61±0.07 | 0.79±0.06 | 0.65±0.10 |
| Bogalusa | N | 98 | 13 | 128 | 15 | 254 |
| Adolescence | age, y | 14.8±1.6 | 14.9±1.9 | 14.8±1.5 | 14.7±1.3 | 15.3±1.5 |
| | LDL-C, mmol/L | 2.39±0.72 | 2.62±0.67 | 2.48±0.61 | 2.76±0.67 | 2.47±0.66 |
| | HDL-C, mmol/L | 1.44±0.52 | 1.17±0.54 | 1.56±0.49 | 1.32±0.39 | 1.48±0.51 |
| Adulthood | age, y | 31.8±1.5 | 32.2±1.6 | 31.9±1.5 | 31.9±1.2 | 32.4±1.4 |
| | LDL-C, mmol/L | 3.21±0.81 | 3.50±0.66 | 3.03±0.89 | 3.39±0.83 | 3.15±0.85 |
| | HDL-C, mmol/L | 1.09±0.27 | 1.15±0.37 | 1.28±0.35 | 1.22±0.49 | 1.20±0.34 |
| | IMT, mm | 0.73±0.11 | 1.06±0.11 | 0.67±0.09 | 0.92±0.08 | 0.73±0.14 |
| CDAH | N | 119 | 24 | 131 | 12 | 286 |
| Adolescence | age, y | 13.4±1.5 | 13.1±1.5 | 13.4±1.5 | 13.3±1.5 | 13.4±1.5 |
| | LDL-C, mmol/L | 2.61±0.56 | 2.73±0.67 | 2.67±0.63 | 3.58±1.58 | 2.69±0.69 |
| | HDL-C, mmol/L | 1.38±0.26 | 1.45±0.35 | 1.50±0.28 | 1.36±0.38 | 1.44±0.29 |
| Adulthood | age, y | 33.4±1.7 | 32.9±1.6 | 33.4±1.6 | 32.9±1.8 | 33.3±1.7 |
| | LDL-C, mmol/L | 3.21±0.71 | 3.26±0.75 | 2.79±0.80 | 3.96±2.17 | 3.05±0.90 |
| | HDL-C, mmol/L | 1.27±0.29 | 1.33±0.30 | 1.57±0.36 | 1.57±0.33 | 1.43±0.36 |
| | IMT, mm | 0.60±0.08 | 0.80±0.08 | 0.59±0.07 | 0.78±0.05 | 0.62±0.10 |

Statistics are means ± SD.

To convert LDL-C and HDL-C to mg/dl, multiply values by 38.67.

### 5.4.3 COMPARISON OF NCEP VS. NHANES CUT-POINTS

The prevalence of adolescent LDL cholesterol dyslipidemia was 32.3% for NCEP or 19.6% for NHANES cut-points. HDL cholesterol dyslipidemia was prevalent in 2.5% adolescents using NCEP cut-points or 6.1% adolescents using NHANES cut-points. Pooled data showed that the risk of having high IMT in adulthood was significantly higher in those with borderline- and high-risk LDL cholesterol levels in adolescence compared with those with normal levels using NHANES cut-points (Figure 39A). Use of the NCEP LDL cholesterol cut-points showed only those with high-risk levels in adolescence had significantly increased risk of high IMT in adulthood compared with those classified as normal, although the borderline-risk group showed a non-significant trend toward increased risk (Figure 39A). A graded increase in the risk of having high IMT in adulthood was observed when moving from the normal, to borderline-risk, to high-risk groups for both NCEP and NHANES LDL cholesterol cut-points (Figure 39A).

Normal weight adolescents with NCEP classified high-risk (low) HDL cholesterol were at significantly increased risk of having IMT at or above the 90$^{th}$ percentile at follow-up (Figure 39B). There was no increased risk of having high IMT at follow-up for normal weight adolescents classified as borderline-risk HDL cholesterol levels using either classification or for those with high-risk HDL cholesterol levels according to NHANES cut-points (Figure 39B). For overweight or obese adolescents, the risk of high IMT in adulthood was significantly increased in those with borderline- or high-risk HDL cholesterol levels compared with those who had normal levels using either paediatric classification (Figure 39C). The relative risks were similar for overweight or obese adolescents with borderline- *or* high-risk HDL cholesterol levels. When data where not stratified by BMI status, only those with high-risk HDL cholesterol were at significantly increased risk of having high IMT at follow-up (NCEP, relative risk= 2.5, 95%CI 1.4-4.6; NHANES, relative risk = 1.6, 95%CI 1.0-2.7).

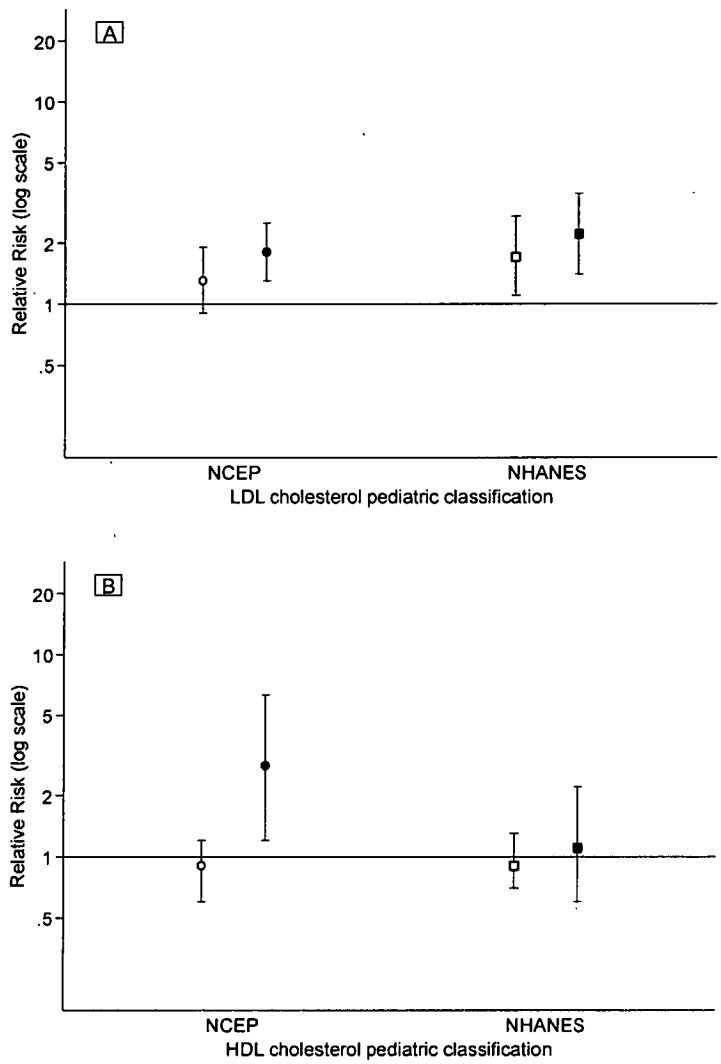


**Figure 39.** Adjusted (age, sex, cohort, length of follow-up) relative risks and 95% confidence intervals for having high intima-media thickness (≥90$^{th}$ percentile) in adulthood according to NCEP and NHANES cut-points for: A) LDL cholesterol dyslipidemia status, B) HDL cholesterol dyslipidemia status in normal weight adolescents, and C) HDL cholesterol dyslipidemia status in overweight/obese adolescents.

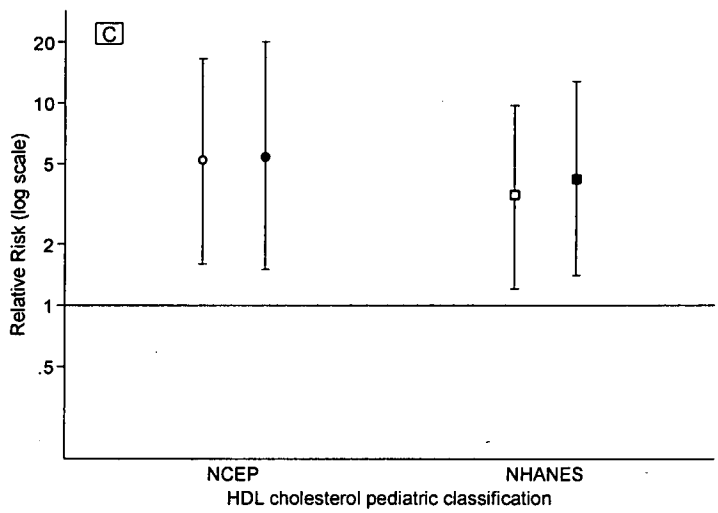


**Figure 39 (con't)**. Adjusted (age, sex, cohort, length of follow-up) relative risks and 95% confidence intervals for having high intima-media thickness ($\geq 90^{th}$ percentile) in adulthood according to NCEP and NHANES cut-points for: A) LDL cholesterol dyslipidemia status, B) HDL cholesterol dyslipidemia status in normal weight adolescents, and C) HDL cholesterol dyslipidemia status in overweight/obese adolescents.

Diagnostic performance statistics comparing NCEP and NHANES classifications in normal weight and overweight or obese adolescents are presented in Table 25. The NCEP high-risk LDL cholesterol cut-points were more sensitive but less specific than the NHANES cut-points. Although this trend in sensitivity and specificity was consistent across cohorts, heterogeneity in the point estimates between cohorts was observed (Appendix 11). False positives were high for both LDL cholesterol classifications with 85.1% of those with high levels using the NCEP cut-points not developing high IMT in young adulthood, and 84.7% with high levels using the NHANES cut-points not having high IMT in young adulthood. Sensitivity, positive predictive value, and AUC values increased when borderline- and high-risk HDL cholesterol cut-points were applied to overweight or obese adolescents (Table 25), but remained low overall. The diagnostic performance characteristics were similar for both classifications of adolescent HDL cholesterol. Area under the receiver operating characteristic curve using other age-, sex-, and cohort-specific cut-points of IMT ($70^{th}$, $75^{th}$, $80^{th}$, $85^{th}$, $90^{th}$, $95^{th}$ percentiles) in these data did not appreciably modify the results presented (Figure 40). Diagnostic statistics of adolescents with other risk factors including hypertension and cigarette smoking in addition to the NCEP dyslipidemia cut-points are displayed in Table 26. The addition of hypertension to the LDL cholesterol cut-points improved the prediction of those with high IMT in adulthood. The prediction was comparable to those who were overweight and did not improve further when either hypertension *or* overweight status was combined with the LDL cholesterol cut-points.

**Table 25.** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) data of NCEP and NHANES classifications for adolescent borderline risk and high risk lipoprotein variable cut-points to predict high carotid IMT in adulthood

| | | Dyslipidemia status in adolescence | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Borderline risk | | | | High risk | | | | | |
| Lipoprotein variable | Classification | Sensitivity | Specificity | PPV | NPV | Sensitivity | Specificity | PPV | NPV | AUC* | P-value† |
| Normal weight | | | | | | | | | | | |
| LDL cholesterol | NCEP | 64.8‡ | 44.0 | 12.2 | 91.3 | 38.9‡ | 68.7 | 12.9 | 90.4 | 0.55 | |
| | NHANES | 50.6 | 56.6‡ | 12.2 | 90.6 | 23.5 | 80.7‡ | 12.7 | 89.8 | 0.55 | 0.73 |
| HDL cholesterol | NCEP | 53.7 | 43.9 | 10.3 | 88.8 | 3.1 | 98.8‡ | 22.7 | 89.5 | 0.49 | |
| | NHANES | 52.5 | 47.0‡ | 10.6 | 89.2 | 4.9 | 95.4 | 11.4 | 89.4 | 0.49 | 0.69 |
| Overweight/obese | | | | | | | | | | | |
| LDL cholesterol | NCEP | 63.2‡ | 52.0 | 24.7 | 84.9 | 50.0‡ | 70.4 | 29.7 | 84.9 | 0.60 | |
| | NHANES | 55.3 | 59.2‡ | 25.3 | 84.1 | 34.2 | 85.5‡ | 37.1 | 83.4 | 0.63 | 0.33 |
| HDL cholesterol | NCEP | 92.1 | 30.5 | 24.6 | 94.0 | 15.8 | 90.3‡ | 28.6 | 81.3 | 0.62 | |
| | NHANES | 89.5 | 30.5 | 24.1 | 92.2 | 26.3 | 83.8 | 28.6 | 82.2 | 0.62 | 0.88 |

Sensitivity = true positives/(true positives + false negatives) X 100. Specificity = true negatives/(true negatives + false positives) X 100. PPV = true positives/(true positives + false positives) X 100. NPV = true negatives/(true negatives + false negatives) X 100.

*Normal-, borderline-, and high-risk included in AUC analyses as three groups.

†Test for difference between AUCs

‡McNemar's test for difference between sensitivities and specificities

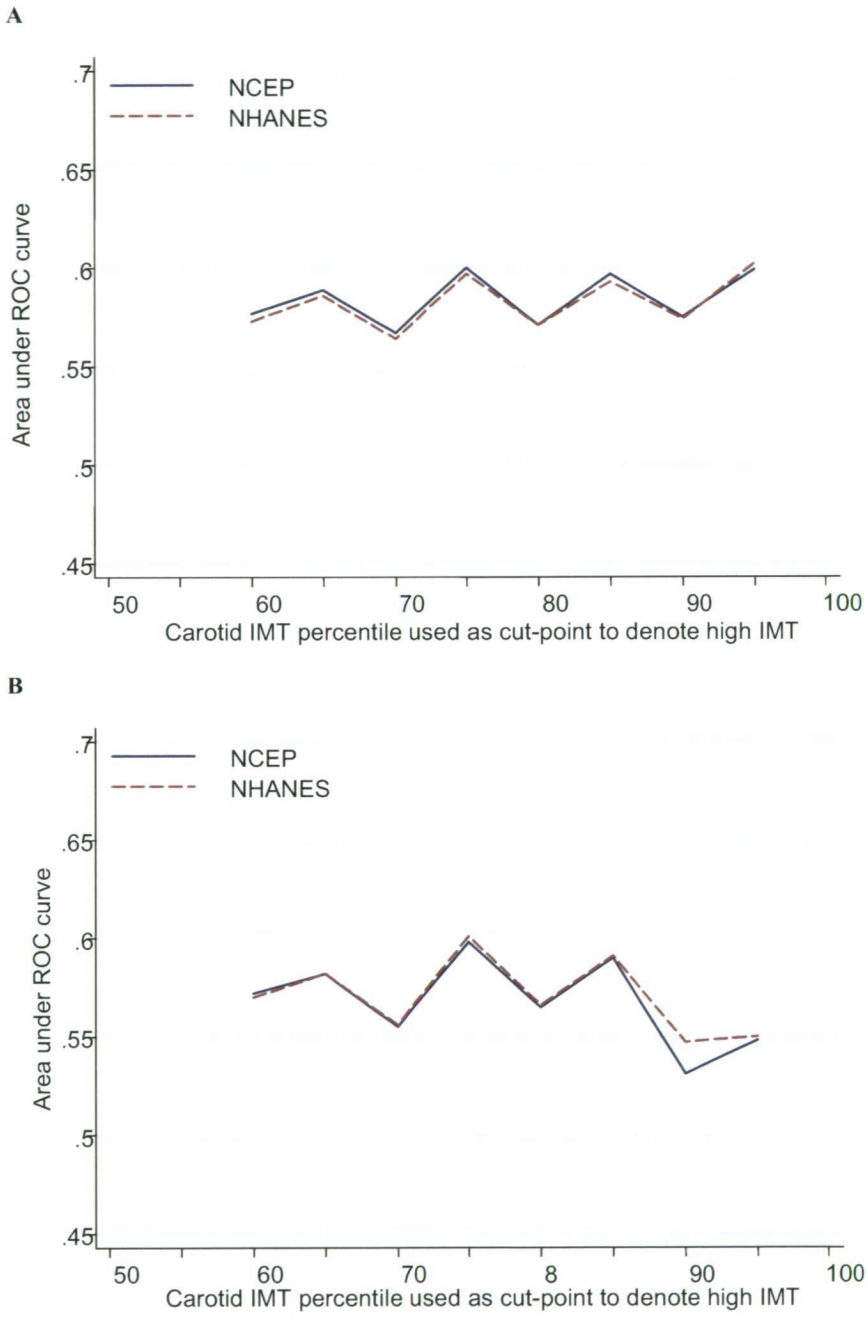


**Figure 40.** Comparison of AUC for the ability of NCEP and NHANES LDL cholesterol (A) and HDL cholesterol (B) dyslipidemia classifications to predict high adult carotid IMT using different percentile cut-points.

**Table 26.** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) data for combining NCEP high risk lipoprotein variable cut-points to other risk factors present in adolescence to predict high carotid IMT in adulthood

| Lipoprotein variable | Screening strategy | N* | Sensitivity | Specificity | PPV | NPV | AUC |
|---|---|---|---|---|---|---|---|
| LDL cholesterol | | | | | | | |
| | Universal | 1707 | 41.0 | 68.9 | 14.9 | 89.8 | 0.56 |
| | Overweight† + NCEP cut-points | 190 | 50.0 | 70.4 | 29.7 | 84.9 | 0.60 |
| | Hypertension‡ + NCEP cut-points | 355 | 45.3 | 71.2 | 21.6 | 88.1 | 0.61 |
| | Smoking§ + NCEP cut-points | 218 | 30.3 | 79.5 | 20.8 | 86.5 | 0.54 |
| | Hypertension† OR Overweight‡ + NCEP cut-points | 491 | 43.4 | 71.6 | 21.9 | 87.4 | 0.60 |
| HDL cholesterol | | | | | | | |
| | Universal | 1710 | 5.5 | 97.8 | 25.6 | 88.7 | 0.53 |
| | Overweight† + NCEP cut-points | 192 | 15.8 | 90.3 | 28.6 | 81.3 | 0.62 |
| | Hypertension‡ + NCEP cut-points | 355 | 7.6 | 99.3 | 66.7 | 86.0 | 0.54 |
| | Smoking§ + NCEP cut-points | 218 | 9.1 | 96.8 | 33.3 | 85.6 | 0.52 |

*Refers to the total number of participants that would be screened; †overweight or obese according to International Obesity Task Force BMI cut-points; ‡hypertension defined according to the National High Blood Pressure Education Program,[305] §Participants were classified as smokers at baseline if they indicated regular cigarette smoking on a weekly basis or more often. Family history was considered but not included in these analyses owing to divergence in the definitions of positive history between the three cohorts.

### 5.4.4 EFFECT OF CHANGE IN DYSLIPIDEMIA STATUS ON HIGH IMT

For the analyses of change in lipoprotein status between adolescence and adulthood, the NHANES classification was used to assign adolescent LDL cholesterol dyslipidemia status and the NCEP classification was used to assign adolescent HDL cholesterol dyslipidemia status. In Table 27 pooled relative risks of having a high IMT in young adulthood according to LDL cholesterol and HDL cholesterol dyslipidemia status during adolescence and adulthood are presented. A change in LDL cholesterol status from non-dyslipidemia to dyslipidemia or dyslipidemia to non-dyslipidemia did not significantly increase the risk of high IMT compared with those who remained normal at both times. Those with LDL cholesterol dyslipidemia at both times had significantly increased risk of high IMT in adulthood than those with persistently normal levels. Analysis of continuous data showed that participants with higher baseline levels of LDL cholesterol had higher carotid IMT as adults (regression coefficient for a one SD increase = 0.014, P<0.001). There was some evidence that those who increased LDL cholesterol levels between adolescence and adulthood had higher carotid IMT at follow-up but this effect was not significant (regression coefficient for a one SD increase = 0.005, P=0.08).

For HDL cholesterol, the relative risk of having a high IMT in adulthood was significantly increased for participants who had low (dyslipidemic) levels in adolescence irrespective of their adult levels (Table 27). The relative risk of high IMT in the non-dyslipidemia to dyslipidemia group was similar to that of the persistent non-dyslipidemia group. Only a small number of participants moved from dyslipidemia to non-dyslipidemia, so the data for this group should be interpreted with caution. Analyses of continuous data showed evidence that higher HDL cholesterol levels in overweight or obese adolescents were associated with lower IMT in adulthood (regression coefficient for a one SD increase = -0.011, P=0.01), whereas change in HDL cholesterol between adolescence and adulthood was not. In normal weight adolescents, analyses of continuous data showed no association between baseline or change in HDL cholesterol levels and carotid IMT in adulthood. In multivariable analyses that included adolescent and change variables for LDL cholesterol and HDL cholesterol in the model, baseline lipoprotein levels were generally stronger predictors of IMT than changes in levels between adolescence and adulthood (Figure 41).

**Table 27.** Relative risks (RR) from pooled data for the effect of change in LDL cholesterol and change in HDL cholesterol status between adolescence and adulthood on carotid artery intima-media thickness ≥90$^{th}$ percentile.

| | Model 1 | | | | Model 2 | | | |
|---|---|---|---|---|---|---|---|---|
| Lipoprotein status in adolescence and adulthood | n/N* | % | RR | 95%CI | n/N*† | % | RR | 95%CI |
| LDL cholesterol | | | | | | | | |
| Persistent non-dyslipidemia | 127/1206 | 10.5 | 1.0 | ref | 121/1148 | 10.5 | 1.0 | ref |
| Non-dyslipidemia to dyslipidemia | 19/143 | 13.3 | 1.3 | 0.8-2.1 | 16/136 | 11.8 | 1.2 | 0.8-1.9 |
| Dyslipidemia to non-dyslipidemia | 25/205 | 12.2 | 1.2 | 0.8-1.9 | 23/191 | 12.0 | 1.2 | 0.7-1.9 |
| Persistent dyslipidemia | 25/124 | 20.2 | 2.1 | 1.4-3.1 | 24/117 | 20.5 | 2.0 | 1.4-3.0 |
| HDL cholesterol | | | | | | | | |
| Persistent non-dyslipidemia | 143/1303 | 11.0 | 1.0 | ref | 135/1234 | 10.9 | 1.0 | ref |
| Non-dyslipidemia to dyslipidemia | 45/358 | 12.6 | 1.2 | 0.9-1.7 | 41/338 | 12.1 | 1.2 | 0.8-1.6 |
| Dyslipidemia to non-dyslipidemia | 4/16 | 25.0 | 2.6 | 1.0-6.5 | 4/15 | 26.7 | 2.8 | 1.1-6.9 |
| Persistent dyslipidemia | 7/27 | 25.9 | 2.6 | 1.3-5.2 | 7/27 | 25.9 | 2.1 | 1.1-4.3 |

Model 1: adjusted for age at baseline, sex, cohort, and length of follow-up

Model 2: additionally adjusted for baseline BMI, baseline smoking status, and baseline systolic blood pressure

* Number of participants with high IMT/all participants.

† Sample sizes differ between models 1 and 2 owing to missing baseline data on smoking and/or systolic blood pressure in some participants.

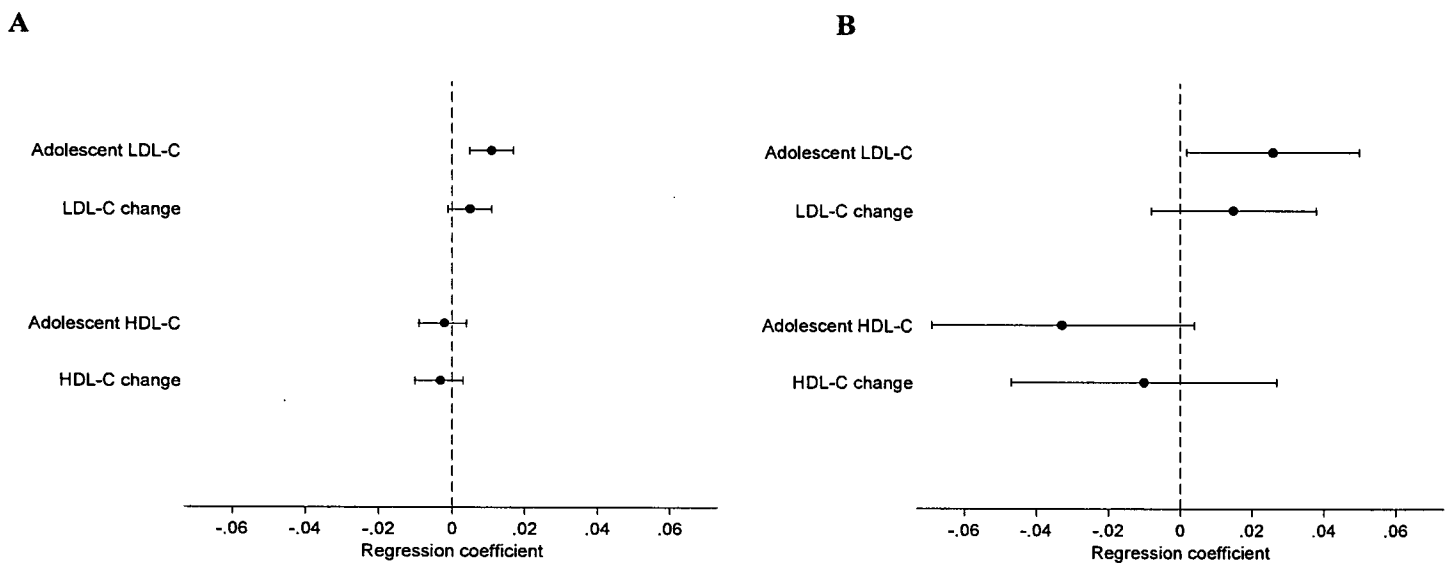


**Figure 41.** Regression coefficients (adjusted for age at baseline, sex, cohort, length of follow-up, systolic blood pressure at baseline, and smoking status at baseline) and their 95% confidence intervals for associations of adolescent LDL-C, change in LDL-C between adolescence and adulthood, adolescent HDL-C, and change in HDL-C with carotid IMT in adulthood for (A) normal weight and (B) overweight or obese adolescents. Regression coefficients expressed in millimetres for a one SD change in the continuous variables.

Based on this model, the level of IMT at 35 years of age for normal weight and overweight or obese adolescents, with normal and dyslipidemic levels of LDL cholesterol and HDL cholesterol at age 15 years was predicted (Figure 42). The difference in IMT at age 35 years for normal-weight adolescents with and without dyslipidemia was minimal. IMT at age 35 years for those who were overweight or obese as adolescents and did not have dyslipidemia were comparable with normal weight adolescents. Those who were overweight or obese and had dyslipidemia as adolescents had markedly higher carotid IMT values at age 35 years than the other groups (Figure 42).

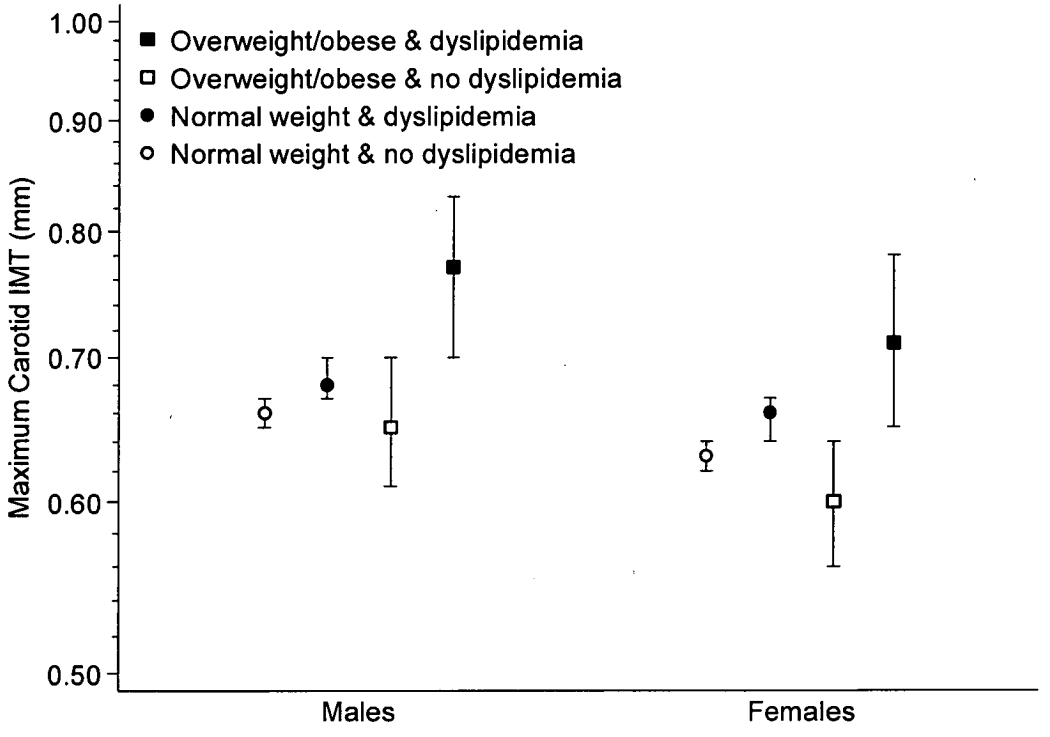


**Figure 42.** Least square means and 95% confidence intervals of carotid IMT at age 35 years for males and females by BMI status and dyslipidemia status at age 15 years, adjusted for length of follow-up, cohort, change in LDL-C, change in HDL-C, baseline smoking status, and baseline systolic blood pressure.

## 5.5. DISCUSSION

The aim of this study was to determine whether two classifications of paediatric dyslipidemia (NCEP vs. NHANES) predicted carotid IMT in adulthood and which of these would be most effective in identifying adolescents at high risk of increased IMT as adults. While these data suggest that both dyslipidemia cut-points were able to predict those with high IMT in adulthood, they do not provide a clear indication of which LDL cholesterol and HDL cholesterol cut-points should be preferred by those who wish to identify adolescents with lipid disorders. Given that there were no substantial differences in the diagnostic performance of the classifications, the simplicity in applying the fixed NCEP cut-points may offer an advantage over the age- and sex-specific NHANES cut-points in a clinical or research setting. Importantly, users of either classification need to be mindful that most adolescents identified with dyslipidemic LDL cholesterol and HDL cholesterol levels will not be at or above the 90$^{th}$ percentile for carotid IMT in early adulthood and most individuals with high IMT will not be identified by using lipoprotein levels in adolescence. Discrimination was considerably better for overweight or obese adolescents compared with their normal-weight peers, which does support targeted, rather than universal (whole- or random-population), screening strategies endorsed in current guidelines.[196, 220, 249]

Single adolescent measures of LDL cholesterol and HDL cholesterol in this study were stronger predictors of adult IMT than change between adolescence and adulthood. While a beneficial change in lipoprotein levels did show trends towards lower IMT in adulthood, particularly for those who were overweight or obese as adolescents, the magnitude of this effect was smaller than for the baseline measure. Moreover, based on predictive modelling (Figure 3), the degree of IMT difference at age 35 years between overweight or obese 15 year olds with LDL cholesterol dyslipidemia and HDL cholesterol dyslipidemia compared with normal-weight 15 year olds with normal LDL cholesterol and HDL cholesterol levels was clinically significant[375] (males, 0.11 mm; females, 0.08 mm) and more than would be expected from measurement error alone.

Taken together, these data and others[35, 37, 41] clearly show the importance of adolescent lipid levels as a predictor of preclinical atherosclerosis and provide some epidemiological rationale for recent recommendations for paediatric lipid screening released in July 2008.[220] However, the efficacy of screening for dyslipidemia in a general population using NCEP or NHANES classifications as a way to identify adolescents at-risk of CVD has considerable limitations (as indicated above), particularly for clinic use. For normal weight

adolescents, population-wide primary prevention programs that target LDL cholesterol levels, such as those used in the Dietary Intervention Study in Children (DISC) and STRIP studies,[163, 164] and maintenance of healthy weight may be the most practical. Lipid screening in the general paediatric population could be limited to adolescents who have other CVD risk factors such as obesity or hypertension to further stratify the risk of this group.

### 5.5.1 LIMITATIONS

This study has a number of potential limitations. First, although the most similar carotid IMT definition was selected, there was heterogeneity in the IMT location and ultrasound protocols between cohorts. While an attempt was made to take this heterogeneity into account by defining high IMT according to age, sex, race (Bogalusa), and cohort specific values, it is noted that attempts by future investigators to merge imaging studies for statistical power will have this limitation unless calls for standardisation of these methods are heeded.[375] Second, one explanation for the poor diagnostic performance of the dyslipidemia classifications in this study may have been due to differing methods of lipoprotein determination between NCEP/NHANES samples and the cohorts used in this study. Lipoprotein levels, particularly HDL cholesterol, have been shown to differ depending on the methodology used.[369] Third, change in dyslipidemia status or change in continuous lipoprotein levels had small, non-significant effects on carotid IMT in adulthood. Because data were only available from two time-points separated by 15 to 20 years, multiple changes that may have occurred in the intervening period or the timing of these changes could not be accounted for. Moreover, multiple lipoprotein measurements were not collected on participants at baseline or follow-up. Use of only one lipoprotein measurement at each time point may have contributed to misclassification of some participants, [161, 308] which would likely lead to an underestimate of the prediction estimates in this study.[337] To improve accuracy, current paediatric guidelines require multiple measurements before lipid levels are classified.[196, 220] Fourth, the potential role of pubertal status as a confounding variable was unable to be accounted for because data were not available for all cohorts. Age was included in these analyses as a proxy for pubertal status given the relationship between sexual maturation and lipids, and age and lipids have been shown to be similar in adolescents.[300] In sensitivity analyses performed using Young Finns data that adjusted for pubertal status, the coefficients were generally unchanged from those analyses where age adjustment was used. Fifth, poorer image quality in ultrasound scans (assessed subjectively by image readers in the Young Finns and CDAH studies) was

associated with BMI (Young Finns, r = 0.12; CDAH, r = 0.18) and had the potential to bias carotid IMT measurements in overweight or obese individuals. Sensitivity analyses adjusting for image quality in the Young Finns and CDAH cohorts yielded similar results to those presented. This issue is covered in detail for the Young Finns and CDAH cohorts in original contribution VI. Briefly, no differences (bias) in the association between BMI and carotid IMT by image quality was observed. Finally, these data are from population-based cohorts and may not be generalised to other higher-risk patients, such as those with diabetes mellitus or a strong family history of premature CVD.

## 5.6. CONCLUSIONS

The data presented in this study indicate that dyslipidemic lipoprotein levels in adolescence are associated with an increased risk of high IMT in young adulthood; that the predictive capacity of both NCEP and NHANES cut-points are similar and could be applied for lipid screening with equal success; that adolescent lipid levels are more strongly associated with high IMT in adulthood than change in lipid levels; and that dyslipidemia in the presence of overweight or obesity, places affected adolescents at substantially higher risk of increased IMT as adults compared with those who do not have both risk factors. These data underscore the importance of both population-wide and individualised prevention programs to improve paediatric dyslipidemia related causes of early atherosclerosis.

**KEY POINTS**

- The aims of this chapter were to determine which of the NCEP or NHANES LDL cholesterol and HDL cholesterol classifications of dyslipidemia status in adolescents is most effective at predicting high common carotid IMT in adulthood; and to assess whether maintaining or changing dyslipidemia status from adolescence to adulthood has an effect on carotid IMT measured in adulthood.

- The findings indicated that paediatric dyslipidemia classifications predict adolescents at increased risk of high IMT in young adulthood; that these classifications perform with equal success; that adolescent lipid levels are more strongly associated with high IMT in adulthood than change in lipid levels; and that dyslipidemia in the presence of overweight or obesity, places affected adolescents at substantially higher risk of increased IMT as adults compared with those who do not have both risk factors.

- The findings underscore the importance of both population-wide and individualised prevention programs to improve paediatric dyslipidemia related causes of early atherosclerosis.

**Box 5.** Summary of key points from Chapter 5: Association of paediatric dyslipidemia classifications and change in dyslipidemia status with carotid IMT in adulthood

# 6. SUMMARY, RECOMMENDATIONS, AND CONCLUSIONS

## 6.1. INTRODUCTION

In this thesis, previous evidence demonstrating the origin of atherosclerosis to childhood and the role of adverse blood lipid and lipoprotein levels in the development of atherosclerosis have been presented. As a result of this evidence, blood lipid and lipoprotein levels have been the target of consensus statements for paediatric screening and treatment issued by the NCEP,[196] the AAP,[220, 247] and the AHA[249] since 1992 (the most recent update being July 2008 from the AAP[220]) and will be an important component in the yet to be released Pediatric Cardiovascular Risk Reduction Initiative by the NHLBI.[380] The basis of these documents is that treatment of lipid disorders beginning in childhood or adolescence may reduce the lifetime risk of CVD. Since the initial publication of the 1992 consensus document, a number of studies have supported and challenged several aspects of the guidelines, while new findings have revealed important gaps in the guidelines. Although interest and concern for the identification and treatment of lipid and lipoprotein abnormalities in the paediatric setting has been intense since the NCEP guidelines were issued, there has been a recent and noticeable gain in momentum towards recognising the limitations of these guidelines and the re-issue of them based on data that have become available in the past 15 years. The rapid gain in adiposity in the paediatric setting since the 1980s is also likely to have contributed to the increased momentum. While a number of areas for revision have been suggested,[249-252] Daniels outlined some of the most important questions concerning the identification of youth with elevated lipid or lipoprotein levels that included: (1) the utility of paediatric lipid and lipoprotein levels for the prediction of important adult outcomes; (2) the best approach (universal vs. targeted) to screening; and (3) the acceptability of using a single set of cut-points for lipids and lipoproteins across all ages as.[381] The aims examined and the data presented in this report make significant progress toward addressing these concerns and as such, progresses the evidence base needed for recommendations on lipid and lipoprotein screening in the paediatric setting. The following sections provide a summary of the key findings from this report, the clinical and public health implications of the findings, and areas for future research.

## 6.2. SUMMARY OF RESULTS

The key findings from this report are listed below, with the novelty of the findings indicated in parentheses:

1. Paediatric classifications for dyslipidemia predict dyslipidemia and arterial thickening in young adulthood 15 to 20 years later (*strengthening evidence*); that the classifications examined differ somewhat in how strongly they predict adult dyslipidemia but perform with equal success in the prediction of arterial thickening in adulthood (*research firsts*);
2. Universal (population-wide) or selective screening approaches that use paediatric dyslipidemia classifications to predict adult dyslipidemia (*strengthening evidence*) and high carotid IMT (*research first*) appear to be limited by either high rates of false positives or high rates of false negatives;
3. Adolescent lipid and lipoprotein levels are more strongly associated with arterial thickening in adulthood than levels measured at the time of the ultrasound examination or change in levels between childhood and adulthood (*strengthening evidence*);
4. Dyslipidemia in the presence of overweight or obesity places affected adolescents at substantially higher risk of increased arterial thickening as adults (*research first*);
5. Unhealthy lifestyle changes that occur between childhood or adolescence and adulthood have an impact on whether an individual maintains, loses, or develops high-risk blood lipid and lipoprotein levels in adulthood (*strengthening evidence*).

## 6.3. CLINICAL AND PUBLIC HEALTH IMPLICATIONS

The findings from this study indicate that there is a progressive and substantial increased risk of adult dyslipidemia and arterial thickening associated with borderline-, and high-risk blood lipid and lipoprotein levels defined according to either of the paediatric dyslipidemia classifications (NCEP or NHANES) examined. The importance of paediatric dyslipidemia in the development of atherosclerosis was further emphasised by data indicating that elevated LDL cholesterol and HDL cholesterol in adolescence were stronger predictors of adult carotid artery thickening than contemporary levels or the change in levels between childhood and adulthood. While the presence of a statistical association between paediatric dyslipidemia and adult outcomes is an important pre-condition of risk factor screening, it is only one component in effective clinical prediction. The diagnostic data presented in this study highlighted possible limitations to strategies for screening in the paediatric setting. First,

while the statistical association was mostly similar across cohorts, there were important differences between cohorts. For example, sensitivity and positive predictive values using LDL cholesterol cut-points were considerably higher in the Young Finns cohort in predicting adult outcomes (dyslipidemia and high IMT) compared with the CDAH and Bogalusa cohorts. For HDL cholesterol, a higher proportion of Bogalusa adults with low HDL cholesterol levels or high IMT would have been identified in childhood compared with the proportions of CDAH or Young Finns adults. These findings suggest that the relative value (prediction, benefits, and adverse effects such as labelling and associated monetary costs) of screening using either paediatric classifications of dyslipidemia may differ by population.

Second, the findings did not provide a clear indication as to whether a universal or targeted approach to screening would optimise the identification of adults with dyslipidemia or high carotid IMT. In these data, both screening approaches were undermined by either unacceptably high rates of false positives or high rates of false negatives. Most individuals that develop low HDL cholesterol levels as adults or high carotid IMT would not be identified in adolescence using either approach, with selective screening further limited by a substantial number of adults with total or LDL cholesterol dyslipidemia not being identified for lipid or lipoprotein screening as adolescents. The inclusion of family history, overweight and obesity, hypertension, smoking, or combinations of these only modestly improved the prediction of dyslipidemia or high IMT in adulthood. This means that clinicians employing current paediatric guidelines for targeted lipoprotein screening in children and adolescents with a positive family history of premature CHD or who are overweight or obese, need to consider that a substantial number of those adolescents identified with high total cholesterol or LDL cholesterol levels will not have high-risk levels or high carotid IMT in early adulthood, that most individuals that develop abnormal HDL cholesterol or triglyceride levels as adults will not be identified in adolescence.

While it is evident that additional data are needed on how to best develop screening strategies for lipid and lipoprotein levels, the inclusion of additional risk factors such as overweight and obesity, hypertension, and smoking did improve, albeit modestly, the performance of targeted lipid and lipoprotein screening. Moreover, it appears that the single set of cut-points issued in the original NCEP guidelines work as well overall as an approach based on age- and sex-distributions, with the simplicity in applying the fixed NCEP cut-points offering a distinct advantage over percentile-based cut-points in a clinical or research setting.

In the debate of routine screening for lipid and lipoprotein disorders to identify youth at substantially increased risk of future CVD, it is important to consider the relationship between lipid and lipoprotein levels in atherosclerosis and the total burden of the disease. For example, because CVD is a disease of mass occurrence, effective programs and interventions implemented at the population level would serve to reduce CVD burden in its entirety. Evidence for effective population-based programs and interventions delivered in the paediatric setting is growing yet not well established. Theoretically however, even small changes in the distribution of lipid and lipoprotein levels and other CVD risk factors at the population level would likely have a large public health impact.[382] Put into context, while the debate of routine screening to identify only those youth at highest risk of subsequent CVD remains an important concern; it is only one component in an effective strategy toward reducing the overall burden of CVD. Admittedly, both population-wide and individualised approaches have been central components of the initial and updated guidelines, but lacking are cost-benefit analyses concerning screening approaches as well as individualised and population-wide intervention programs. This is especially important as new guidelines are developed.

Findings from this study confirmed the importance of adiposity changes on tracking of blood lipid and lipoprotein levels found in the Beaver County and Cardiovascular Risk in Young Finns studies,[223, 224, 235] but also suggested for the first time that changing fitness and socioeconomic circumstance in the time between youth and adulthood may also affect tracking independent of adiposity. Findings for both these factors were limited to HDL cholesterol, which supports the need for other studies to replicate these findings. Importantly though, these data suggest that effective programs and policies that aim to first limit adiposity gains (beyond what is associated with normal growth and development) as well as increase physical activity or cardiorespiratory fitness and socioeconomic conditions may improve adult levels of blood lipid and lipoprotein irrespective of baseline levels. Population-based programs may be best to achieve these aims, but the efficacy of such interventions in the paediatric setting to limit or prevent obesity has not been convincing. For example, a Cochrane review of school-, community-, and family-based interventions on this subject concluded that diet and/or physical activity approaches did not significantly improve BMI levels, despite observed improvements in diet and/or physical activity levels.[383] A more recent review from the same authors on school-based interventions concluded that despite inconsistent trends across studies, the data suggested that combined physical activity-diet

interventions may help prevent children becoming overweight in the long term.[384] While these data underscore the need for more research into effective, population-wide interventions for the prevention and reduction of paediatric obesity, the point made above remains, that even small changes to the population distribution may have a large public health impact.[382] Whether this effect would be large enough to affect lipid and lipoprotein tracking remains untested. Data on the effectiveness of population-based interventions to improve physical activity and fitness in the paediatric setting have been more forthcoming;[385, 386] while interventions to improve socioeconomic circumstance are more difficult. Because the indicator of SEP used in this study was based on education, interventions that seek improvements in education represent one potential strategy through which improvements in SEP may be achieved.[348] Again, the effectiveness of such interventions to achieve the necessary effect on tracking of lipid and lipoprotein levels is not known.

Taken together, the findings highlight that youth lipid and lipoprotein levels are important in predicting adult outcomes, that they are subject to modification, and while there are potential limitations to lipid and lipoprotein screening in the paediatric setting, the data underscore the importance of both population-wide and individualised prevention programs to reduce the early development of atherosclerosis associated with paediatric dyslipidemia.

## 6.4. FUTURE RESEARCH NEEDS

In compiling this work, a number of areas for future research direction and consideration have become evident. A number of these areas have been highlighted in the previous section, but other priority areas include:

- **Factors that affect tracking of blood lipid and lipoprotein levels**: A substantial limitation in this study was the lack of consistent dietary information at both time points to provide meaningful data on the effect of change in a number of important dietary variables (including saturated fat, poly and mono unsaturated fats, cholesterol, and fibre) on lipid and lipoprotein tracking. Thus, this represents one area for future examination. Further, the effect of accelerated adiposity gain during childhood and adolescence on blood lipid and lipoprotein tracking needs to be examined. Because this study only had data available from a single time-point in youth, it was not possible to determine the rapidity of adiposity gain and the subsequent short- and long-term effects on lipid and

lipoprotein tracking. Low birth weight has been associated with more adverse lipid profiles in childhood and adulthood and with a faster yearly rate of LDL change (increase) in the Bogalusa Heart Study suggesting that there may be early *programming* of lipid and lipoprotein levels.[387] The effect of birth weight on lipid and lipoprotein tracking has not been examined however.

- **Systematic review and meta-analysis of studies that have examined tracking of blood lipid and lipoprotein levels between youth and adulthood**: Although over 20 publications from 10 studies have provided data on lipid and lipoprotein tracking from youth into adulthood, only few systematic reviews are available and no meta-analysis has been conducted. Data are available for a good number of participants, over varying lengths of follow-up, from different industrialised populations worldwide, on both males and females, and beginning at a wide range of ages during youth. A meta-analysis of these data would be useful both to summarise findings from these studies that have accumulated over a period of 25 years, and to determine what characteristics may influence tracking. Additionally, it would serve to identify additional areas for future research and to stimulate a new wave of research within the area of lipid and lipoprotein tracking.

- **Multiple blood lipid and lipoprotein measures at baseline and follow-up examinations**: A well known yet woefully unaccounted for fact in study designs, is the measurement variability of lipids and lipoproteins. All guidelines issued on paediatric lipid screening have recognised, and advocated for, multiple measures before risk classification. While this has a considerable cost limitation, studies specifically designed to assess either the efficacy of lipid screening or interventions to improve lipid levels in paediatric populations that do not collect multiple measures at baseline and follow-up could be underestimating the predictiveness of the screening program or missing effects of interventions due to misclassification. This is particularly important for studies with short follow-up periods, where meaningful but small changes may not be able to be detected because they are obscured by measurement error.

- **Cut-points or screening that observe race and maturation differences**: None of the paediatric lipid cut-points examined in this thesis, nor the ones advocated in the July 2008 update of the guidelines for paediatric lipid screening,[220] take account for variations in lipid and lipoprotein levels due to race or pubertal status. Evidence has shown that both race and pubertal status have a substantial influence on blood lipid and lipoprotein levels.[300, 388-391] One wonders given the limitations observed in this study concerning high rates of false-positives and false-negatives whether the prediction of adult CVD risk, subclinical-, or clinical disease may improve if cut-points observed stage of sexual maturation, race, *and* sex. Another consideration may be to avoid lipid and lipoprotein screening altogether during the pubertal years. The LRC data provided in the Friedman[276] paper showed that prediction of adult total and LDL cholesterol levels from levels measured in youth was poorest during the years of puberty. An approach that incorporates screening before and after puberty may reduce the proportions of false positive and false negative individuals.

- **Utility of non-HDL cholesterol levels for paediatric screening**: Non-HDL cholesterol, obtained directly by subtracting HDL cholesterol levels from total cholesterol levels, is considered by some groups as a simpler and more effective screening tool for the assessment of CVD risk compared with LDL cholesterol. Non-HDL measurements avert certain limitations of LDL cholesterol measurements estimated using the Friedewald equation.[314] For example, estimated LDL cholesterol does not include all classes of atherogenic lipoproteins, and the measurement does not require overnight fasting.[392] Consequently, non-HDL cholesterol is increasingly being used in clinical research involving adult populations,[392, 393] and has also been specified as a secondary target for therapy among patients with the metabolic syndrome or diabetes mellitus in the NCEP ATPIII recommendations.[304] For paediatric populations, past and current screening guidelines have not advocated for the measurement of non-HDL cholesterol. This is puzzling considering available data that have shown: child non-HDL cholesterol to be a better predictor of adult dyslipidemia and non-lipid CVD risk factors in adulthood such as hyperinsulinemia, and hyperglycaemia compared with LDL cholesterol;[234] non-HDL to be strongly predictive of metabolic related risk factors such as triglycerides, HDL cholesterol, adiposity, and $HBA_{1c}$ in childhood;[394, 395] youth with type 1 and type 2 diabetes to commonly have elevated non-HDL cholesterol,[396, 397] and that the prevalence

of high non-HDL cholesterol levels in youth with type 1 diabetes increases with poor glycaemic control and duration of diabetes.[395] Non-HDL cholesterol is also included in the calculation of the PDAY risk score.[47] These and other available evidence suggest that childhood non-HDL cholesterol levels have a place in the prediction of dyslipidemia and nonlipid CVD risk factors (particularly metabolic-related abnormalities) in adulthood. Future research should aim to establish paediatric cut-points for non-HDL cholesterol; or should cut-points for non-HDL cholesterol be included in the upcoming release from the NHLBI's Expert Panel for the Pediatric Cardiovascular Risk Reduction Initiative, the cut-points and associated screening procedures should be subject to similar predictive testing that other lipid and lipoproteins have been submitted to.

- **Utility of paediatric blood lipid and lipoprotein levels to predict CVD morbidity and mortality**: Data linking childhood and adolescent blood lipid and lipoprotein levels to clinically important CVD outcomes is currently lacking. The cohorts used in this study will likely be the first to be able to provide these data, but with the oldest participants in each of these samples still only in their fifth decade of life, a time when CVD is only just beginning to present, it appears these important analyses are still some time off. The collaboration between the three cohorts used in this thesis may provide an avenue for performing these analyses sooner by pooling event data, which was the main goal underlying the partnership between the groups. Funding to determine the feasibility of such analyses using these three cohorts has recently been granted (Magnussen CG, from the Finnish Foundation for Cardiovascular Research, May 2009). These data will be important to determine the causal role of blood lipid and lipoprotein levels in youth in the development of clinically significant disease.

- **Dichotomous vs. weighted scoring systems for lipid and CVD risk assessment**: An ongoing concern surrounding dichotomous definitions for CVD risk factors (both in adult and paediatric settings) is that lipid and lipoprotein levels span a continuum of risk, where specific inflection points may not exist and the interplay of them with other CVD risk factors such as obesity, blood pressure, and smoking are not taken into consideration. This may be particularly important if an interaction between lipid and lipoprotein levels and other related risk factors may lead to exponential rather than additive increases in risk. An example from this study is evident in the data presented in Chapter 5, where the

presence of overweight and obesity status in addition to high-risk LDL and HDL cholesterol levels in adolescence substantially increased adult IMT levels. It is also possible that different thresholds exist where more aggressive treatment or intervention may be required. These problems have also been outlined as distinct limitations of the dichotomous definition of the metabolic syndrome, with calls from a recent Scientific Statement from the AHA calling for the development of a weighted scoring system that takes into account each risk factor, their interaction, and important patient characteristics.[127] In line with such an approach for paediatric metabolic syndrome, a weighted scoring system that takes into account the full spectrum of risk associated with increasing lipid and lipoprotein levels, and indeed other CVD risk indicators, may also prove useful toward improved identification and risk stratification of youth with elevated lipid and lipoprotein levels or elevated CVD risk.

- **Tools for estimating absolute CVD risk and the possibility of risk assessment without laboratory analysis in the paediatric setting**: While lipid and lipoprotein levels are an important contributor to CVD, they are not the only contributor. Multiple, mostly preventable, causal factors contribute to CVD.[398] The probability that an individual will develop CVD within a given period depends more closely on the presence of a number of risk factors rather than any single risk factor.[399] That is, the screening for and possible modification of, multiple CVD risk factors may be more effective in reducing CVD risk than a major reduction of a single risk factor.[400] Because of the available evidence, a number of countries now adopt adult guidelines based on the assessment and treatment of *absolute* CVD risk rather than separate guidelines that focus on individual risk factors.[401-403] Given the evidence available in the paediatric setting suggesting the importance of a number of risk factors in the prediction of atherosclerosis in pathological and imaging studies, a risk approach that considers the assessment of multiple risk factors appears pertinent. The development, validation, and calibration of such risk scores in the paediatric setting are therefore required. Because data on clinical outcomes are not available, preclinical markers may be a useful alternative as an intermediary outcome in the development and testing of these scores. Should such a tool be developed for use in the paediatric setting, approaches that simplify risk assessment but maintain adequate risk prediction should be examined. In the adult setting, a recent paper by Gaziano et al.[404] demonstrated that non-laboratory-based measures (age, sex,

smoking, blood pressure, adiposity) included in a risk score that omitted a blood draw (and subsequent lipid and lipoprotein analysis) performed equally in the prediction of CVD events compared with non-laboratory- plus laboratory-based measures. It has also been shown that a simple lifestyle score derived from eight factors (normal BMI, non-smoking, low alcohol, salt and meat consumption and regular fish consumption, leisure time physical activity and skim milk use) predicts mortality in elderly men[405, 406] and cardiometabolic risk factors in young adult males and females aged 25 to 36 years.[407] A simplified risk assessment or score that could omit a blood draw and provide reasonable discrimination has obvious advantages in the paediatric setting.

## 6.5. CONCLUSIONS

The findings from this study suggest that paediatric dyslipidemia classifications are useful in predicting adolescents who are at increased risk of having dyslipidemia or preclinical atherosclerosis in young adulthood. While the findings highlight potential limitations to lipid and lipoprotein screening in the paediatric setting, they underscore the importance of both population-wide and individualised prevention programs to reduce the early development of atherosclerosis associated with paediatric dyslipidemia.

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# APPENDICES

# APPENDIX 1

## Non-invasive imaging techniques used to assess preclinical atherosclerosis

**Table.** Summary of non-invasive imaging techniques used to assess preclinical atherosclerosis

| | Arterial Phenotype | | | |
|---|---|---|---|---|
| | Wall thickness (structural)[60, 61] | Endothelial function (functional)[62, 63] | Elasticity/stiffness/distensibility (mechanical)[64, 65] | Calcification (structural) |
| Premise | -Diffuse intimal thickening occurs early in atherosclerosis, prior to lesion development. | -The endothelium is the key regulator of vascular homeostasis and has antithrombotic and antiproliferative properties. The presence of nitric oxide (NO) in the endothelium plays a major role in both actions.<br><br>-Alteration in endothelial function precedes structural atherosclerotic changes. | -Proximal large arteries have high elastic properties that are a result of high elastin to collagen ratio in their walls. The gradual loss of elastic properties of these variables with aging is a result of progressive elastic fibre degeneration.<br><br>-Decreased elasticity is thought to represent an early risk marker for CVD. | -Coronary artery calcification is part of the pathogenesis of atherosclerosis.<br><br>-It occurs almost exclusively in atherosclerotic arteries.<br><br>-Is absent in the normal vessel wall. |
| Technique | -High-resolution B-mode ultrasonography of large (carotid, aorta, femoral) and conduit (brachial) arteries to assess the intima-media thickness (distance between the lumen-intima interface and the media-adventitia interface).<br><br>-IMT measurements can be quantified as the average of arterial wall thickness (excluding segments involved with plaque), the maximal measured value, and as the average of multiple values measured at different sites/segments. | -High-resolution B-mode ultrasonography to measure diameter of artery before and in response to shear stress (increased blood flow) during reactive hyperaemia (induced by inflation and then deflation of a blood pressure cuff around the forearm). This technique is known as brachial artery flow-mediated dilatation (FMD).<br><br>-The degree of FMD occurs mainly due to NO release from the endothelium.<br><br>-Decreased FMD or the absence of a response is suggestive of endothelial dysfunction. | Various indices and techniques used. These include:<br>-Arterial wave propagation (Pulse-wave velocity, PWV between two points, e.g. carotid-femoral).<br><br>-Carotid & aortic pressure waves (central pulse & systolic blood pressures, augmentation index).<br><br>-Change in vessel diameter to distending pressure (compliance, distensibility) | -Detected by cardiac computed tomography: electron-beam CT (EBCT) & multi-detector CT (MDCT).<br><br>- Both technologies employ thin-slice CT imaging (1.5-6 mm slice thickness), using fast scan speeds to reduce motion artefact.<br><br>-Scans are obtained with participants in a supine position during 1 or 2 breath-holding sequences and are triggered by continuously measured ECG at 40% to 80% of the R-R interval (near end-diastole before atrial contraction), to minimise the effect of cardiac motion.[408]<br><br>-Presence and degree of calcium is scored according to 2 methods (Agatson and volume), with the summed (total) score for the entire coronary arteries typically used.<br><br>-The amount of calcium detected |

| | Arterial Phenotype | | | |
|---|---|---|---|---|
| | Wall thickness (structural)[60, 61] | Endothelial function (functional)[62, 63] | Elasticity/stiffness/distensibility (mechanical)[64, 65] | Calcification (structural) |
| | | | | indicates the amount of underlying coronary atherosclerosis. |
| Limitations | -Imaging equipment is not currently able to differentiate between the intima and media, so both are measured. Variation in IMT measurements may be due to hypertrophy of media not thickening of intima.<br><br>-Internal carotid and near walls cannot be measured precisely in a large number of subjects.<br><br>-Heterogeneity in protocols (imaging, measurements, arterial segment) used introduce difficulties in pooling data from multiple studies and for meta-analysis.<br><br>-Imperfect correlation with coronary atherosclerosis. | -Requires skilled sonographer and appropriate training period.<br><br>-High biological variability (reflected by wide normal-range of dilatation response).<br><br>-Not high test-retest repeatability (product of high biological variability and within- and between-measurer differences).<br><br>-Degree of dilatation can be effected by: recent smoking, recent food/beverage intake (high fat meal, alcohol, vitamin C), recent exercise, circadian pattern, viral illness, and phase of the menstrual cycle for females.<br><br>-Imperfect correlation to coronary circulation.<br><br>-Vasodilatory response is related to vessel size, suggestions that those with brachial arteries ≥4.7-mm should have FMD of radial artery examined. | <u>PWV</u><br>-Does not provide information on arterial geometry.<br><br>-Inaccuracy of distance measurement.<br><br><u>Central pulse-waves</u><br>-Indirect measure of arterial stiffness.<br><br><u>Change in vessel diameter to distending pressure</u><br>-Requires skilled technician<br><br>-Requires measurement of central pulse pressure. | -Radiation exposure<br><br>-High rates of zero scores before 5th decade of life.<br><br>-'Noise' can be mistaken for calcium on CT images leading to higher calcium scores, particularly in those with large chest size or those with high body mass index.<br><br>-Cost<br><br>-More recent technologies (smaller slice thicknesses, higher speed, prospective cardiac gating) have been found to have high reproducibility, but earlier technologies were shown to have poor short-term test-retest reproducibility.[66]<br><br>-Vulnerable plaque may not exhibit significant calcification. |
| Predictive value | -Largest amount of evidence available for common carotid IMT.<br><br>-A recent systematic review and meta-analysis demonstrated that for each 0.1mm increase in common carotid IMT, the risk of MI increased by 15% and the risk of stroke by 18%.[375] | -Attenuated FMD has been shown to be an independent predictor of CVD events in those with established atherosclerosis. More recently, Yeboah et al. demonstrated the prognostic utility of FMD in a large prospective study of older adults without established CVD.[409] | <u>PWV</u><br>-Gold-standard measure of arterial stiffness.<br><br>-Largest amount of evidence available.<br><br>-Has independent predictive value for CVD morbidity and mortality.<br><br><u>Central pulse-waves</u><br>-Independent predictors of all-cause mortality and CVD events persons with established CVD.<br><br>-No data available from disease-free populations.<br><br><u>Change in vessel diameter to distending pressure</u> | -No meta-analysis available, but there is a high level of evidence demonstrating the presence and extent of coronary calcium to predict cardiac events in both symptomatic and asymptomatic individuals.[410]<br><br>-Effect tends to remain after adjustment for other (more conventional) risk factors and there are data to suggest the addition of |

| | Arterial Phenotype | | | |
|---|---|---|---|---|
| | Wall thickness (structural)[60, 61] | Endothelial function (functional)[62, 63] | Elasticity/stiffness/distensibility (mechanical)[64, 65] | Calcification (structural) |
| | | | -Evidence suggestive of independent association with CVD events in those with established CVD, although the independent effect has been challenged.<br><br>-No data available from disease-free populations. | coronary calcium to risk scores improves CVD event prediction, particularly in those with intermediate 10-year risk.[411] |
| Simplicity* | ++ | + | ++/+++/+ | + |
| Reproducibility* | +++ | + | ++/++/+ | ++ |
| Safety* | +++ | +++ | +++/+++/+++ | + (radiation) |
| Low cost* | ++ | ++ | +++/+++/++ | + |

* Ratings based on review articles of these measures.[60, 63-67] + = fair, ++ = good, +++ = excellent

# APPENDIX 2

## CDAH Enrolment Questionnaire

# ENROLMENT QUESTIONNAIRE


Your completion of this Enrolment Questionnaire will allow us to confirm your personal details and enrol you as a study participant in the Childhood Determinants of Adult Health (CDAH) Study. At each later stage of follow-up, you will be provided with additional study information and given a further opportunity to consent or decline to participate in that stage.

**Instructions for completing questionnaire:**
***Please write in BLOCK LETTERS using a black pen (if possible).***
***Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the boxes or space.***

Shade circles like this ●
Not like this ⊘

## Your Personal Details

**Name:**

First Name*

Middle Name(s)

Preferred Name
(if different to *)

Surname

**Home Address:**

Suburb

Postcode

**Postal Address:**
**(if different)**

Suburb

Postcode

**Telephone Numbers:**

Home – Work –

Mobile –

Email

**Your Date of Birth:** / /

**Your Marital Status:**

| | | | |
|---|---|---|---|
| Single | O | Separated/Divorced | O |
| Married | O | Widowed | O |
| De facto | O | Other | O |

**Which school were you at in 1985?**

**What is the highest level of formal education that you have completed?**

| | |
|---|---|
| Primary School | O |
| Grade 7-9 | O |
| Grade 10 | O |
| Grade 11 | O |
| Grade 12 | O |
| Trade Certificate | O |
| Technical College | O |
| Undergraduate university studies | O |
| Postgraduate university studies | O |

**What is your current employment status?**

| | |
|---|---|
| Employed full-time | O |
| Employed part-time or casual | O |
| Unemployed | O |
| Home duties | O |
| Student | O |
| Permanently unable to work / Disabled | O |

**How tall are you?** ☐☐☐ cm OR ☐☐ ft ☐☐ in

**How much do you weigh?** ☐☐☐ kg OR ☐☐ st ☐☐ lb

**Have you ever been a regular smoker?** Yes O No O

*(A regular smoker is someone who has smoked at least 7 cigarettes, cigars or pipes every week for at least 3 months)*

**Are you currently a regular smoker?** Yes O No O

**In general, would you say your health is:**

| | | | |
|---|---|---|---|
| Excellent | O | Fair | O |
| Very Good | O | Poor | O |
| Good | O | | |

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**The name and contact details of two people who will always know where you are if you move:**

**CONTACT 1**

**First Name:**

**Surname:**

What is their relationship to you?

Parent O Grandparent O Brother/Sister O Other relative O Friend O Other O

If other, please specify

**Address:**

Suburb

Postcode

**Telephone Numbers:**

Home - Work -

Mobile -

Email

**CONTACT 2**

**First Name:**

**Surname:**

What is their relationship to you?

Parent O Grandparent O Brother/Sister O Other relative O Friend O Other O

If other, please specify

**Address:**

Suburb

Postcode

**Telephone Numbers:**

Home - Work -

Mobile -

Email

# APPENDIX 3

## CDAH Short Questionnaire

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| SCAN | VERIFY | CHECK |
|---|---|---|
| | | |

Participant ID

# CDAH SHORT QUESTIONNAIRE

## GENERAL INFORMATION

1. How tall are you? ___ cm OR ___ ft ___ in

2. (Females only) Are you currently pregnant? O Yes ⟶ Go to question 4
   O No

3. How much do you weigh? ___ kg OR ___ st ___ lb

4. Which of the following describes your current employment status? (You can pick more than one).
   - O Working full-time
   - O Working part-time
   - O Not working (but not retired)
   - O Home duties
   - O Full-time student
   - O Part-time student
   - O Retired
   - O Permanently unable to work / Ill
   - O Other _____ *(please specify)*

5. What is the highest level of education you have completed? (Select only one answer)
   - O Primary School
   - O Year 7, 8 or 9 or equivalent
   - O Year 10 or equivalent
   - O Year 11 or equivalent
   - O Year 12 or equivalent
   - O Trade/apprenticeship (e.g. hairdresser, chef)
   - O Certificate/diploma (e.g. child care, technician)
   - O University Degree
   - O Higher University Degree (e.g. Grad Dip, Masters, PhD)
   - O Other _____ *(please specify)*

6. What is your current marital status?
   - O Single
   - O Married
   - O De facto
   - O Separated/Divorced
   - O Widowed
   - O Other _____ *(please specify)*

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## HEALTH AND LIFESTYLE

7. Has a doctor or nurse ever told you that you have diabetes? No O ---> Go to question 8
Yes O

IF 'YES'

7a) In what year were you first told that you had diabetes? ☐☐☐☐ Year

7b) Were you told that you had: (select one)

Type 1 diabetes (previously known as "insulin-dependent diabetes") O

Type 2 diabetes (previously known as "non insulin-dependent diabetes") O

Don't know which type O

7c) What advice and/or treatment have you had for diabetes? (select all that apply)

Diet advice O

Tablets O

Insulin injections O

Diet advice and tablets O

Diet advice and insulin injections O

8. Over your lifetime, have you smoked at least 100 cigarettes, or a similar amount of tobacco?
No O ---> Go to question 10
Yes O

IF 'YES'

9. Have you ever been a daily smoker? No O ---> Go to question 10
Yes O

9a) When did you start smoking daily? ☐☐ Years of Age
OR ☐☐☐☐ Year

9b) How often do you now smoke cigarettes, cigars, pipes or any other tobacco products?

Daily O ---> Go to question 10

At least once a week (but not daily) O

Less often than weekly O

Not at all O

9c). When did you finally stop smoking daily? ☐☐ Years of Age
OR ☐☐☐☐ Year

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10. How often, on average, have you have consumed an alcoholic drink in the last 12 months? This includes light beer, medium strength beer, full strength beer, wine, champagne, sparkling wine, wine cooler, spirit-based mixed drinks like Lemon Ruski, sherry, port or fortified wines, spirits, liqueurs, and other alcoholic drinks like cider. Please choose from the following options:

| | |
|---|---|
| Never, or less than once a month | O |
| 1-3 times per month | O |
| Once per week | O |
| 2-4 times per week | O |
| 5-6 times per week | O |
| Once per day | O |
| 2-3 times per day | O |
| 4-5 times per day | O |
| 6 or more times per day | O |

**MALES:** —> Go to page 4

**FEMALES ONLY:**

11. Have you ever been pregnant?

Yes O

No O —> Go to page 4

11a). How many times have you been pregnant? ☐☐ times

11b). How many live births have you had? ☐☐

11c). When were these babies born?

| | Baby | Month | Year |
|---|---|---|---|
| First baby | 1 | ☐☐ | ☐☐☐☐ |
| Second baby | 2 | ☐☐ | ☐☐☐☐ |
| Third baby | 3 | ☐☐ | ☐☐☐☐ |
| Fourth baby | 4 | ☐☐ | ☐☐☐☐ |
| Fifth baby | 5 | ☐☐ | ☐☐☐☐ |

*(If more than 5 live births, please record in empty space below)*

11d). Were you ever told that you had gestational diabetes or pregnancy related diabetes?

Yes O

No O

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## PHYSICAL ACTIVITY

**We are interested in finding out about the kinds of physical activities that people do as part of their daily lives. The following questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.**

Think about all the **vigorous** activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those activities that you did for at least 10 minutes at a time.

12. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging aerobics or fast bicycling?

☐ **days per week**

O **No vigorous physical activities** —> **Skip to question 13**

12a). How much time did you usually spend on one of those days doing vigorous physical activities?

☐☐ **hours** ☐☐ **minutes** **Per day** O **Don't know/Not sure**

Think about all the **moderate** activities that you did in the last 7 days. Moderate physical activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those activities that you did for at least 10 minutes at a time.

13. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace or doubles tennis? Do not include walking.

☐ **days per week**

O **No moderate physical activities** —> **Skip to question 14**

13a). How much time did you usually spend on one of those days doing **moderate** physical activities?

☐☐ **hours** ☐☐ **minutes** **Per day** O **Don't know/Not sure**

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place and any other walking that you might do soley for recreation, sport , exercise or leisure.

14. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

☐ **days per week**

O **No walking** —> **Skip to question 15**

14a). How much time did you spend walking on one of those days walking?

☐☐ **hours** ☐☐ **minutes** **Per day** O **Don't know/Not sure**

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Think about the time you spend sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.

15. During the last 7 days, how much time did you usually spend sitting on a weekday?

☐☐ hours ☐☐ minutes Per day O Don't know/Not sure

## YOUR HEALTH NOW

16. In general would you say your health is now:

O Excellent O Very Good O Good O Fair O Poor

17. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

| | YES, limited a lot | YES, limited a little | NO, not limited at all |
|---|---|---|---|
| 17a) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf. | O | O | O |
| 17b) Climbing several flights of stairs. | O | O | O |

18. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 18a) Accomplished less than you would like | O | O | O | O | O |
| 18b)Were limited in the <u>kind</u> of work or other activities. | O | O | O | O | O |

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19. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 19a) Accomplished less than you would like | O | O | O | O | O |
| 19b) Did work or other activities less carefully than usual | O | O | O | O | O |

20. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

| Not at all | A little bit | Moderately | Quite a bit | Extremely |
|---|---|---|---|---|
| O | O | O | O | O |

21. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

| How much of the time during the past 4 weeks: | All of the time | Much of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 21a) Have you felt calm and peaceful? | O | O | O | O | O |
| 21b) Did you have a lot of energy? | O | O | O | O | O |
| 21c) Have you felt downhearted and depressed? | O | O | O | O | O |

22. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.)?

| All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|
| O | O | O | O | O |

**This is the end of the questionnaire, thank you for participating.**

# APPENDIX 4

## CDAH General Questionnaire

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| SCAN | VERIFY | CHECK |
|---|---|---|
| | | |

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(Bar code ID here)

**SECTION A:** This section asks you some questions about yourself. You may feel you have already answered these questions in our enrolment questionnaire, however your circumstances may have changed since you completed our enrolment questionnaire.

1. **Todays date** ☐☐ / ☐☐ / ☐☐

2. **What sex are you?** O Male O Female

3. **What is your date of birth?** ☐☐ / ☐☐ / ☐☐

4. **What is your current marital status?**

- O Single
- O Married
- O De facto
- O Separated/Divorced
- O Widowed
- O Other ☐ (please specify)

5. **What is the <u>highest</u> level of education you have completed? (Select only one answer)**

- O Primary School
- O Year 7, 8 or 9 or equivalent
- O Year 10 or equivalent
- O Year 11 or equivalent
- O Year 12 or equivalent
- O Trade/apprenticeship (e.g. hairdresser, chef)
- O Certificate/diploma (e.g. child care, technician)
- O University Degree
- O Higher University Degree (e.g. Grad Dip, Masters, PhD)
- O Other ☐ (please specify)

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6. **What is your <u>main</u> source of income? (Select only one answer)**

- O Wages or salary
- O Own business or share in partnership
- O A government pension or cash benefit
- O Superannuation
- O Investment/ Interest
- O Other income ☐ (please specify)

7. **What is your <u>main</u> occupation NOW (Select only one answer)**

Manager or administrator (e.g. magistrate, farm manager, general manager, director of nursing, school principal) ---- O

Professional (e.g. scientist, doctor, registered nurse, allied health professional, teacher, artist) ---- O

Associate professional (e.g. technician, manager, youth worker, police officer) ---- O

Tradesperson or related worker (e.g. hairdresser, gardener, florist) ---- O

Advanced clerical or service worker (e.g. secretary, personal assistant, flight attendant, law clerk) ---- O

Intermediate clerical, sales or service worker (e.g. typist, word processing/data entry operator, receptionist, child care worker, nursing assistant, hospitality worker) ---- O

Intermediate production or transport worker (e.g. sewing machinist, machine operator, bus driver) ---- O

Elementary clerical, sales or service worker (e.g. filing/mail clerk, parking inspector, sales assistant, telemarketer, housekeeper) ---- O

Labourer or related worker (e.g. cleaner, factory worker, general farm hand, kitchen hand) ---- O

No paid job ---- O

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8. **Which of the following describes your current employment status? You can pick more than one.**

- O Working full-time
- O Working part-time
- O Not working (but not retired)
- O Home duties
- O Full-time student
- O Part-time student
- O Retired
- O Permanently unable to work / Ill
- O Other [ ] (please specify)

## SECTION B: This section is about your health and your medical history

1. **Have you <u>ever</u> been told that you have high blood pressure?**

O No -->**Skip to Question 2**

O Yes

**<u>IF 'YES'</u>**

**1a) When were you first told this?** [ ][ ][ ][ ] (Year)

**1b) Was this during pregnancy?** O Yes O No O Not applicable

**1c) Are you currently taking medication prescribed by a doctor to lower your blood pressure?**

O Yes O No

**1d) Has a doctor in the past year recommended you change your way of life, in order to lower your blood pressure?**

O Yes O No

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2. Have you ever been told that you have angina?

O No -->Skip to Question 3

O Yes

IF 'YES'

2a) When were you first told this? ☐☐☐☐ (Year)

2b) Are you currently on tablets or other treatment for angina?

O Yes O No

3. Have you ever been told that you have had a heart attack (includes 'coronary', 'coronary occlusion', 'coronary thrombosis, 'myocardial infarction')?

O No -->Skip to Question 4

O Yes

IF 'YES'

3a) When were you first told this? ☐☐☐☐ (Year)

4. Have you ever been told that you have had a stroke?

O No -->Skip to Question 5

O Yes

IF 'YES'

4a) When were you first told this? ☐☐☐☐ (Year)

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5. Have you <u>ever</u> been told that you have high cholesterol?

O No -->Skip to Question 6

O Yes

<u>IF 'YES'</u>

5a) When were you first told this? ☐☐☐☐ (Year)

5b) Are you currently taking medication prescribed by a doctor to lower your blood cholesterol?

O Yes O No

5c) Has a doctor in the past year recommended that you change your way of life, in order to lower your blood cholesterol?

O Yes O No

6. Have you <u>ever</u> been told that you have high triglycerides?

O No --> Skip to Question 7

O Yes

<u>IF 'YES'</u>

6a) When were you first told this? ☐☐☐☐ (Year)

7. Are you currently taking aspirin-containing medication to prevent or treat heart disease or stroke?

O No

O Yes

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**8 Has a doctor or nurse ever told you that you have diabetes?**

O No --> **Skip to Question 9**

O Yes

**IF 'YES'**

**8a) In what year were you first told that you had diabetes?** ☐☐☐☐ (Year)

**8b) Were you told that you had:**

| | **(select one)** |
|---|---|
| Type 1 diabetes (previously known as "insulin-dependant diabetes") | O |
| Type 2 diabetes (previously known as "non insulin-dependant diabetes") | O |
| Don't know which type | O |

**8c) What advice and/or treatment have you had for diabetes?** *(select all that apply)*

O Diet advice

O Tablets

O Insulin injections

O Diet advice and tablets

O Diet advice and insulin injections

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9. **Are you currently taking any medication prescribed by a doctor?**

O No --> **Skip to Question 9b**

O Yes

9a) **In the table below please provide the name (or type of medication) and what it was prescribed for. Please continue at the bottom of page 28 if you need more space.**

| | *Medication* | *Prescribed for* |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |

9b) **Are you currently using any of the following hormonal medications?**
*(If you are female using hormonal contraceptives please do not include them in the "Other" category. We ask about contraception in Section D)*

O I do not use any hormone medications

O Hormone replacement therapy

O Testosterone treatment (e.g., Androderm)

O Anabolic steroids

O Other (please specify) ☐

10. **Have you had any illness causing a high temperature during the last two weeks?**

O No --> **Skip to SECTION C (Page 8)**

O Yes

**IF 'YES'**

10a) **What was the duration of the fever?** ☐☐ days

10b) **Was your temperature measured?**

O No

O Yes, but temperature not known

O Yes, my temperature was ☐☐.☐ °C

10c) **How many days ago did the fever stop?** ☐☐ Days ago

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**SECTION C:** The following questions ask for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

1. **In general would you say your health is:**

O Excellent O Very Good O Good O Fair O Poor

2. **The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?**

| | YES, limited a lot | YES, limited a little | NO, not limited at all |
|---|---|---|---|
| 2a) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf. | O | O | O |
| 2b) Climbing several flights of stairs. | O | O | O |

3. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 3a) Accomplished less than you would like | O | O | O | O | O |
| 3b) Were limited in the kind of work or other activities. | O | O | O | O | O |

4. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)**

| | All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 4a) Accomplished less than you would like | O | O | O | O | O |
| 4b) Did work or other activities less carefully than usual | O | O | O | O | O |

1849007492 9

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

| Not at all | A little bit | Moderately | Quite a bit | Extremely |
|---|---|---|---|---|
| O | O | O | O | O |

6. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

| How much of the time during the past 4 weeks: | All of the time | Much of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|
| 6a) Have you felt calm and peaceful? | O | O | O | O | O |
| 6b) Did you have a lot of energy? | O | O | O | O | O |
| 6c) Have you felt downhearted and depressed? | O | O | O | O | O |

7. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.)?

| All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|
| O | O | O | O | O |

8369007496 10

## SECTION D: This section is for WOMEN ONLY.

If you are MALE please skip to SECTION E (page 15). The answers to the following questions will help us investigate the influence of hormones on the cardiovascular system.

1. **Are you currently using any of the following hormonal contraceptives, even if you are using them for reasons other than contraception?**

O Oral contraceptive pill

O Minipill (progesterone only pill)

O Weekly contraceptive patch

O Progestagen (e.g., Implanon)

O Progestagen injection (e.g., Depo Provera)

O Progestin injection ( e.g. , Noristerat)

O Progestin releasing intrauterine device ( e.g. , Mirena, Copper T380A)

O Progestin releasing implant ( e.g. , Norplant)

O Other (please specify) ☐

2. **How old were you when you had your first menstrual period?**

Years ☐☐ Months ☐☐

3. **Have you had a hysterectomy; that is, an operation to remove your uterus?**

O No -->**Skip to Question 4**

O Yes

**IF YES**

**3a) What age were you when you had the hysterectomy?** ☐☐ **Years**

**3b) Were your ovaries removed as well?**

O Yes, both ovaries removed

O Yes, only one ovary removed

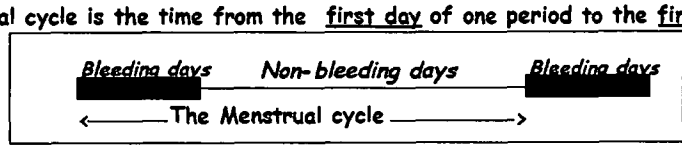
O No

O Don't know

**SKIP TO Question 5**

7072007496 11

4. The menstrual cycle is the time from the first day of one period to the first day of the next.




4a) How long is your usual menstrual cycle?
In other words, how many days are there from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT?

☐☐☐ Days

4b) What is the longest menstrual cycle you have had in the last 12 months? Again count from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT.

☐☐☐ Days

4c) What is the shortest menstrual cycle you have had in the last 12 months? Again count from the FIRST DAY OF ONE PERIOD to the FIRST DAY OF THE NEXT.

☐☐☐ Days

5. Thinking about the most recent time when you were having periods and were NOT using hormonal contraceptives (e.g., the pill) and were not pregnant or breastfeeding:

5a) Would you describe your periods as:

O Very regular
O Fairly regular
O Irregular
O Very irregular

5b) How old were you at this time?
That is, at the most recent time when you were having periods and were NOT using hormonal contraceptives (e.g., the pill) and were not pregnant or breastfeeding.

☐☐ Years

5c) During this time, approximately how many periods did you have in the space of 12 months?

O More than 13
O 11-13
O 6-10
O 1-5
O None

5232007492 ☐☐☐☐ 12

6. Have you ever seen a doctor because of irregular periods?

O No -->Skip to Question 7

O Yes

IF YES

6a) How old were you when you first saw your doctor about irregular periods?

☐☐ Years

6b) Have you ever taken prescribed hormone medications for irregular periods?

O Yes O No

6c) Has a doctor ever told you that you have polycystic ovaries or polycystic ovary syndrome?

O Yes O No

7. Have you ever seen a doctor because of concern about the amount of hair on your face?

O No -->Skip to Question 8

O Yes

IF YES

7a) Were you prescribed any treatment for this?

O No

O Yes ☐ (please specify)

8. Has a doctor ever told you that you have acne?

O No -->Skip to Question 9

O Yes

IF YES

8a) Were you prescribed any treatment for this?

O No

O Yes ☐ (please specify)

9926007496 13

9. **Have you ever tried to become pregnant for 12 months or more without succeeding?**

O Yes O No

10. **Have you ever seen a doctor because you were having trouble becoming pregnant?**

O No -->**Skip to Question 11**

O Yes

**IF YES**

**10a) Did you have any of the following fertility investigations?**

O Test of blood or urine hormone levels

O Laparoscopy (incision in your stomach to look at your reproductive organs)

O Your partner's semen analysed

**10b) Did a doctor ever tell you that you or your partner had:**

O An ovulatory problem?

O A tubal problem?

O Any other female fertility problem?

- please specify

O Semen abnormalities?

O An unexplained fertility problem?

11. **Have you ever been pregnant?**

O No -->**Skip to SECTION E (Page 15)**

O Yes

12. **How many times have you been pregnant?** **times**

3670007496 14

13. How many live births have you had? ☐☐

13a) When were these babies born?

| | Baby | Month | Year |
|---|---|---|---|
| First baby | 1 | ☐☐ | ☐☐☐☐ |
| Second baby | 2 | ☐☐ | ☐☐☐☐ |
| Third baby | 3 | ☐☐ | ☐☐☐☐ |
| Fourth baby | 4 | ☐☐ | ☐☐☐☐ |
| Fifth baby | 5 | ☐☐ | ☐☐☐☐ |

*If you have had more than 5 live births please continue at the end of this section.*

14. When you were pregnant were you ever tested for diabetes?
That is, did you have a blood or urine sugar test? This may have involved drinking a very sugary drink.

O Yes O No

15. Were you ever told that you had gestational diabetes or pregnancy related diabetes?

O Yes O No

9309007491 15

## SECTION E: This section is about your family's medical history

**The following questions are about your BIOLOGICAL parents and siblings. Because heart disease in women under 50 is uncommon, some questions are only asked about your male relatives. Please do not include adoptive or step-parents or siblings here.**

1. **Was your biological mother diagnosed with diabetes when she was under the age of 50?**

O Yes O No O Don't Know

2. **Is your biological mother alive now?** O Yes O No O Don't Know

**If NO:**

**2a) How old was she when she died?** ☐☐ **Years**

**2b) Was the cause of her death diabetes?**

O Yes O No O Don't Know

3. **Was your biological father diagnosed with diabetes when he was under the age of 50?**

O Yes O No O Don't Know

4. **Was your biological father diagnosed with heart disease when he was under the age of 50?**

O Yes O No O Don't Know

5. **Is your biological father alive now?** O Yes O No O Don't Know

**If NO:**

**5a) How old was he when he died?** ☐☐ **Years**

**5b) Was the cause of his death:**

Heart disease? O Yes O No O Don't Know

Diabetes? O Yes O No O Don't Know

0949007492 ☐☐☐ 16

6. Do you have any BIOLOGICAL brothers?

O Yes

O No -->Skip to question 7

O Don't Know -->Skip to question 7

IF YES

6a) Have any of your biological brothers been diagnosed with the following illnesses when under the age of 50?

Heart disease O Yes O No O Don't Know

Diabetes O Yes O No O Don't Know

6b) If 'YES' to either of the above, please complete details below (space has been allowed for you to complete details for up to 3 brothers if necessary):

HEART DISEASE

| | Age at diagnosis (if known) | Did this result in his death? |
|---|---|---|
| 1 | ☐☐ | O Yes O No |
| 2 | ☐☐ | O Yes O No |
| 3 | ☐☐ | O Yes O No |

DIABETES

| | Age at diagnosis (if known) | Did this result in his death? |
|---|---|---|
| 1 | ☐☐ | O Yes O No |
| 2 | ☐☐ | O Yes O No |
| 3 | ☐☐ | O Yes O No |

8784007497 17

7. Do you have any BIOLOGICAL sisters

O Yes

O No -->Skip to SECTION F

O Don't know -->Skip to SECTION F

IF YES

7a) Have any of your biological sisters been diagnosed with diabetes when under the age of 50?

O Yes O No O Don't Know

7b) If 'YES', please complete details below (space has been allowed for you to complete details for up to 3 sisters if necessary):

| | Age at diagnosis (if known) | Did this result in her death? |
|---|---|---|
| 1 | | O Yes O No |
| 2 | | O Yes O No |
| 3 | | O Yes O No |

## SECTION F: This section is about smoking tobacco

1. Over your lifetime, have you smoked at least 100 cigarettes, or a similar amount of tobacco?

O No --> SKIP TO SECTION G (Page 20)

O Yes

2. How often do you now smoke cigarettes, cigars, pipes or any other tobacco products?

O Daily

O At least once a week (but not daily) -->Skip to Question 7

O Less often than weekly -->Skip to Question 7

O Not at all -->Skip to Question 7

1497007491 18

3. When did you start smoking daily? Years of Age ☐☐ OR Year ☐☐☐☐

4. What do you currently smoke?
(Please indicate types and enter how many you smoke)

| | | |
|---|---|---|
| 4a) | O Manufactured cigarettes | ☐☐☐ Cigarettes per day |
| 4b) | O Hand-rolled cigarettes | ☐☐☐ Grams per week* |
| 4c) | O Cigars | ☐☐☐ Cigars per week |
| 4d) | O Pipes full of tobacco | ☐☐☐ Grams per week* |

* A one and three quarter ounce pouch of tobacco equals 50 grams

5. When you smoke manufactured cigarettes, which brand do you usually smoke?

I do not smoke manufactured cigarettes O

The brand I usually smoke is ☐
*(Please give as much detail as possible, eg Marlboro Lights)*

6. Have there been any periods of time when you gave up daily smoking and then started smoking again?

No O -->Skip to SECTION G (Page 20)

Yes O

IF YES

6a) Were any of these periods greater than 3 months duration?

No O -->Skip to SECTION G (Page 20)

Yes O

IF YES 6b) What is the total amount of time that you stopped smoking for?
(Please add together all the periods of time when you stopped smoking)

Years ☐☐ Months ☐☐ Now skip to SECTION G (Page 20)

9793007498 19

7. In the past have you ever been a daily smoker?

No O -->Skip to SECTION G (Page 20)

Yes O

| | Years of Age | | Year | |
|---|---|---|---|---|
| 8. When did you start smoking daily? | | OR | | |
| 9. When did you finally stop smoking daily? | | | | |

10. When you smoked daily, how much did you usually smoke?
(Please indicate types and enter the number smoked)

| | | |
|---|---|---|
| 10a) | O Manufactured cigarettes | Cigarettes per day |
| 10b) | O Hand-rolled cigarettes | Grams per week* |
| 10c) | O Cigars | Cigars per week |
| 10d) | O Pipes full of tobacco | Grams per week* |

* A one and three quarter ounce pouch of tobacco equals 50 grams

11. When you smoked manufactured cigarettes, which brand did you usually smoke?

I did not smoke manufactured cigarettes O

The brand I usually smoked was

(Please give as much detail as possible, eg Marlboro Lights)

12. Prior to the time when you finally stopped daily smoking, were there any periods of time when you gave up daily smoking and then started smoking again?

No O -->Skip to SECTION G (Page 20)

Yes O

IF YES

12a) Were any of these periods greater than 3 months duration?

No O -->Skip to SECTION G (Page 20)

Yes O

IF YES 12b) What is the total amount of time that you stopped smoking for?
(Please add together all the periods of time when you stopped smoking)

Years Months

1012007494 20

**SECTION G:** These questions are about your life when you were growing up until the age of 12. They are mostly about your parents or other adults who you lived with and who were responsible for you.

1. This question is about only the people who lived in the same house as you and were like parents to you for most of the time until you turned 12.

1a) Did you live in the same house as your father or another male who was like a father to you?

O No --> Skip to question 1c

O Yes

IF YES

1b) What is the highest level of education completed by your father (or other male who lived with you and was like a father to you)

O No schooling

O Primary School only

O Year 7, 8 or 9 or equivalent

O Year 10 or equivalent

O Year 11 or equivalent

O Year 12 or equivalent

O Trade/apprenticeship (e.g. hairdresser, chef)

O Certificate/diploma (e.g. child care, technician)

O University Degree

O Higher University Degree (e.g. Grad Dip, Masters, PHD)

O Other (please specify) _____

9189007497 21

1c) **Did you live in the same house as your <u>mother</u> or another female who lived with you and was like a mother to you?**

O No -->**Skip to question 2**

O Yes

**<u>IF YES</u>**

1d) **What is the <u>highest</u> level of education completed by your mother (or other female who lived with you and was like a mother to you)**

O No schooling

O Primary School only

O Year 7, 8 or 9 or equivalent

O Year 10 or equivalent

O Year 11 or equivalent

O Year 12 or equivalent

O Trade/apprenticeship (e.g. hairdresser, chef)

O Certificate/diploma (e.g. child care, technician)

O University Degree

O Higher University Degree (e.g. Grad Dip, Masters, PHD)

O Other (please specify) [ ]

5680007499 22

2. What was the MAIN occupation of your father (or other male who lived with you and was like a father to you), and your mother (or other female who lived with you and was like a mother to you) until you turned 12? *Please only select one answer for your father and one answer for your mother.*

| Occupation | Father | Mother |
|---|---|---|
| Manager or administrator (e.g. magistrate, farm manager, general manager, director of nursing, school principal | O | O |
| Professional (e.g. scientist, doctor, registered nurse, allied health professional, teacher, artist) | O | O |
| Associate professional (e.g. technician, manager, youth worker, police officer) | O | O |
| Tradesperson or related worker (e.g. hairdresser, gardener, florist) | O | O |
| Advanced clerical or service worker (e.g. secretary, personal assistant, flight attendant, law clerk) | O | O |
| Intermediate clerical, sales or service worker (e.g. typist, word processing/ data entry operator, receptionist, child care worker, nursing assistant, hospitality worker) | O | O |
| Intermediate production or transport worker (e.g. sewing machinist, machine operator, bus driver) | O | O |
| Elementary clerical, sales or service worker (e.g. filing/mail clerk, parking inspector, sales assistant, telemarketer, housekeeper) | O | O |
| Labourer or related worker (e.g. cleaner, factory worker, general farm hand, kitchenhand) | O | O |
| No paid job | O | O |

3. Thinking about until you were 12, how many rooms were there in the home where you lived the longest?
*Please include buildings on the property that were regularly used for living, such as bungalows. If your house had open plan areas, consider each area as a separate room (i.e. an open plan kitchen, dining and living area would count as three rooms). Do not include separate toilets.*

☐☐ Rooms in house

0122007495 ☐☐☐ 23

4. **Thinking about most of the years <u>until you were 12</u>, did your parents or the people who brought you up own the house you <u>mostly</u> lived in, or did they rent it?**

O They owned or were paying off the house

O They rented the house

O Unsure

5. **Thinking about the years <u>until you were 12</u>, how many times did you move house? If you did not move house, please write "0"**

☐☐ Times

6. **All together, how many brothers and sisters did you have in your family <u>until you were 12</u>? Include adopted, step and half brothers and sisters.**
***Please also include any brothers or sisters that may have died, but not those who died before you were born.***

I did not have any brothers or sisters O

☐ Older brothers

☐ Older sisters

☐ Younger brothers

☐ Younger sisters

☐ Twin brother to you

☐ Twin sister to you

7. **About how much did you weigh when you were born?**

O 3 pounds or less (less than 1360g)

O More than 3 pounds and up to 5 pounds (1361-2270 grams)

O More than 5 pounds and up to 8 pounds (2271-3630 grams)

O More than 8 pounds (more than 3630 grams)

O Don't know

0688007492 24

**SECTION H:** The following statements have been used by many people to describe how much support they get from other people.

***We would like to know whether you share any of these feelings and how strongly you feel about them, by filling in the circle according to whether you strongly agree, agree, disagree or strongly disagree with each one. If you are undecided, select the column with this heading.***

| | Strongly Agree | Agree | Undecided | Disagree | Strongly Disagree |
|---|---|---|---|---|---|
| 1. People do not come and visit me as often as I would like. | O | O | O | O | O |
| 2. I find it easy to make friends. | O | O | O | O | O |
| 3. I often need help from other people but can't get it. | O | O | O | O | O |
| 4. I'm afraid of being left alone. | O | O | O | O | O |
| 5. I seem to have a lot of friends. | O | O | O | O | O |
| 6. I don't have anyone that I can confide in. | O | O | O | O | O |
| 7. The person who means most to me takes an interest in my affairs. | O | O | O | O | O |
| 8. There is someone who needs me as much as I need them. | O | O | O | O | O |
| 9. I don't have a very close friend. | O | O | O | O | O |
| 10. The person who means most to me does spend time with me. | O | O | O | O | O |
| 11. I have no-one to lean on in times of trouble. | O | O | O | O | O |
| 12. I have someone to share good news with. | O | O | O | O | O |
| 13. There is someone who can always cheer me up. | O | O | O | O | O |
| 14. I often feel very lonely. | O | O | O | O | O |
| 15. I feel there is something missing from my life. | O | O | O | O | O |

7871007493 25

**SECTION I:** This section contains 60 statements. Read each statement carefully. For each statement fill in the circle with the response that best represents your opinion. Make sure your answer is in the correct box.

***Fill in Strongly Disagree if you strongly disagree or the statement is definitely false***
***Fill in Disagree if you disagree or the statement is mostly false***
***Fill in Neutral if you are neutral about the statement, you cannot decide, or the statement is about equally true and false***
***Fill in Agree if you agree or the statement is mostly true***
***Fill in Strongly Agree if you strongly agree or the statement is definitely true***

| | Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree |
|---|---|---|---|---|---|
| 1. I am not a worrier. | O | O | O | O | O |
| 2. I like to have a lot of people around me. | O | O | O | O | O |
| 3. I do not like to waste my time. | O | O | O | O | O |
| 4. I try to be courteous to everyone I meet. | O | O | O | O | O |
| 5. I keep my belongings clean and neat. | O | O | O | O | O |
| 6. I often feel inferior to others. | O | O | O | O | O |
| 7. I laugh easily. | O | O | O | O | O |
| 8. Once I find the right way to do something, I stick to it. | O | O | O | O | O |
| 9. I often get into arguments with my family and co-workers. | O | O | O | O | O |
| 10. I am pretty good about pacing myself so as to get things done on time. | O | O | O | O | O |
| 11. When I am under a great deal of stress, sometimes I feel like I am going to pieces. | O | O | O | O | O |
| 12. I do not consider myself especially "light-hearted". | O | O | O | O | O |
| 13. I am intrigued by the patterns I find in art and nature. | O | O | O | O | O |
| 14. Some people think I am selfish and egotistical. | O | O | O | O | O |
| 15. I am not a very methodical person. | O | O | O | O | O |
| 16. I rarely feel lonely or blue. | O | O | O | O | O |

2686007492 26

| | Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree |
|---|---|---|---|---|---|
| 17. I really enjoy talking to people. | O | O | O | O | O |
| 18. I believe letting students hear controversial speakers can only confuse and mislead them. | O | O | O | O | O |
| 19. I would rather cooperate with others than compete with them. | O | O | O | O | O |
| 20. I try to perform all the tasks assigned to me conscientiously. | O | O | O | O | O |
| 21. I often feel tense and jittery. | O | O | O | O | O |
| 22. I like to be where the action is. | O | O | O | O | O |
| 23. Poetry has little or no effect on me. | O | O | O | O | O |
| 24. I tend to be cynical and sceptical of others' intentions. | O | O | O | O | O |
| 25. I have a clear set of goals and work toward them in an orderly fashion. | O | O | O | O | O |
| 26. Sometimes I feel completely worthless. | O | O | O | O | O |
| 27. I usually prefer to do things alone. | O | O | O | O | O |
| 28. I often try new and foreign foods. | O | O | O | O | O |
| 29. I believe that most people will take advantage of you if you let them. | O | O | O | O | O |
| 30. I waste a lot of time before settling down to work. | O | O | O | O | O |
| 31. I rarely feel fearful or anxious. | O | O | O | O | O |
| 32. I often feel as if I am bursting with energy. | O | O | O | O | O |

5885007496 27

| | Strongly Disagree | Disagree | Undecided | Agree | Strongly Agree |
|---|---|---|---|---|---|
| 33. I seldom notice the moods or feelings that different environments produce. | O | O | O | O | O |
| 34. Most people I know like me. | O | O | O | O | O |
| 35. I work hard to accomplish my goals. | O | O | O | O | O |
| 36. I often get angry at the way people treat me. | O | O | O | O | O |
| 37. I am a cheerful, high-spirited person. | O | O | O | O | O |
| 38. I believe we should look to our religious authorities for decisions on moral issues. | O | O | O | O | O |
| 39. Some people think of me as cold and calculating. | O | O | O | O | O |
| 40. When I make a commitment, I can always be counted on to follow through. | O | O | O | O | O |
| 41. Too often, when things go wrong, I get discouraged and feel like giving up. | O | O | O | O | O |
| 42. I am not a cheerful optimist. | O | O | O | O | O |
| 43. Sometimes when I am reading poetry or looking at a work of art, I feel a chill or wave of excitement. | O | O | O | O | O |
| 44. I am hard-headed and tough-minded in my attitudes. | O | O | O | O | O |
| 45. Sometimes I am not as dependable or reliable as I should be. | O | O | O | O | O |
| 46. I am seldom sad or depressed. | O | O | O | O | O |
| 47. My life is fast-paced. | O | O | O | O | O |
| 48. I have little interest in speculating on the nature of the universe or the human condition. | O | O | O | O | O |
| 49. I generally try to be thoughtful and considerate. | O | O | O | O | O |

5962007492

28

| | Strongly Disagree | Disagree | Undecided | Agree | Strongly Agree |
|---|---|---|---|---|---|
| 50. I am a productive person who always gets the job done. | O | O | O | O | O |
| 51. I often feel helpless and want someone else to solve my problems. | O | O | O | O | O |
| 52. I am a very active person. | O | O | O | O | O |
| 53. I have a lot of intellectual curiosity. | O | O | O | O | O |
| 54. If I do not like people, I let them know it. | O | O | O | O | O |
| 55. I never seem to be able to get organised. | O | O | O | O | O |
| 56. At times I have been so ashamed I just wanted to hide. | O | O | O | O | O |
| 57. I would rather go my own way than be a leader of others. | O | O | O | O | O |
| 58. I often enjoy playing with theories or abstract ideas. | O | O | O | O | O |
| 59. If necessary, I am willing to manipulate people to get what I want. | O | O | O | O | O |
| 60. I strive for excellence in everything I do. | O | O | O | O | O |

# APPENDIX 5

## Definition of smoking status for each cohort

**Current smoking status: Toward a consistent definition between CDAH, Young Finns, and Bogalusa studies.**

Bogalusa:

At baseline (ages 8 to 17y) and follow-up, participants were categorized as:

1) currently smokes at least one cigarette a week (≥1/week)
2) currently experimenting with cigarettes (fewer than one cigarette per week) (<1/week)
3) used to smoke at least one cigarette a week but no longer smokes cigarettes (former)
4) at one time was experimenting (<1/week) with cigarettes but quit smoking (former experimenter)
5) never experimented with cigarettes (never)

Young Finns:

At baseline (those aged 12 years or older) and follow-up, participants were categorized:

1) once per day or more often (≥1/day)
2) at least once per week, but not daily (≥1/week)
3) less than once per week (<1/week)
4) stopped smoking or do not smoke at present (former)
5) never smoked (never)

CDAH:

At baseline, participants aged 9 years and older were asked a series of questions on smoking behaviour that included the following question:

'How long have you been smoking regularly? (regularly means 1 or more times a week)'.

1) 'I don't smoke';
2) 'just started';
3) '1 to 6 months';
4) '7 months to 1 year';
5) '1 to 2 years';
6) '2 to 4 years';
7) 'more than 4 years'.

These responses could be collapsed into a binomial categorical variable indicating:

1) at least once per week (≥1/week), i.e. those that indicated any of options 2-7.
2) less than once per week (<1/week), i.e. those that indicated 'I don't smoke'

Never smokers at baseline could be ascertained from the question:
'Have you ever smoked even part of a cigarette?'. Those responding 'No' could be classified as Never smokers at baseline.

At follow-up, those that indicated they had smoked at least 100 cigarettes or equivalent tobacco over their lifetime were asked the following question:
'How often do you now smoke cigarettes, etc':

1) Daily (≥1/day)
2) At least once a week (but not daily) (≥1/week)
3) Less often than weekly (<1/week)
4) Not at all (Ex-smoker)

Considering the available data from the three cohorts, it appears the most consistent definition of current smoking status that could be applied at both baseline and follow-up would involve collapsing the responses into three categories:

1) Currently smoking at least once per week (≥1/week)
2) Currently smoking less often than weekly (<1/week)
3) Not currently smoking (includes never or former smokers)

# APPENDIX 6

**Correction factors used in the Cardiovascular Risk in Young Finns Study to account for changes in lipid and lipoprotein determination kits between baseline and follow-up surveys**

**Lipid and lipoprotein correction factors used in the Cardiovascular Risk in Young Finns Study**

Due to changes in lipid and lipoprotein determination methods and kits during study years, levels from 1980 were corrected by using the following correction factor equations. These equations were determined by linear regression analysis utilizing standardized principal component adjustments. All equations were developed by the Young Finns research group previously.[218]

Total cholesterol = 1.091 * total cholesterol (1980) – 0.271 mmol/L
HDL cholesterol = 1.068 * HDL cholesterol (1980) – 0.0277 mmol/L
Triglycerides = 1.00758 * triglycerides (1980) + 0.0582 mmol/L

# APPENDIX 7

## Overview of studies of blood lipid and lipoprotein tracking from childhood to adulthood: correlation data

**Table.** Overview of studies that have examined tracking by correlation of blood lipid levels in childhood or adolescence and adulthood, sorted by date of first publication and study

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Analyses (adjustments) | Findings | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | | TCH | LDL-C | HDL-C | TG | |
| Beaver County Lipid Study, USA | Orchard, 1983[235] | 561 | 264 M; 297 F | 9 y | 11-14 y | 20-24 y | 10 h | Correlations – type not specific (adjusted for age) | M: r=0.57<br>F: r=0.49 | NA | NA | NA | |
| | Stuhldreher, 1991[224] | 295 | 144 M; 155 F | 16 y | 11-14 y | 27-30 y | 10 h | Correlations – type not specific (no adjustments specified) | M: r=0.38<br>F: r=0.51 | NA | NA | NA | |
| Muscatine Study, USA | **Lauer, 1988[160]/1989[232]** | **2446** | **1167 M; 1279 F** | **6-15 y** | **8-18 y** | **20-30 y** | **Yes** | **Pearson's correlation coefficients stratified by age & sex** | | NA | NA | NA | Only TCH collected at baseline. Correlations between TCH (baseline) and other lipids at follow-up are presented but not listed here. |
| | | 99 | 55 M; 54 F | 15 y | 7-8 y | 20-25 y | | | M: r=0.56<br>F: r=0.64 | | | | |
| | | 612 | 292 M; 311 F | 13 y | 9-10 y | 20-25 y | | | M: r=0.58<br>F r=0.49 | | | | |
| | | 1018 | 476 M; 542 F | 12 y | 11-12 y | 20-25 y | | | M: r=0.51<br>F: r=0.54 | | | | |
| | | 1041 | 490 M; 551 F | 10 y | 13-14 y | 20-25 y | | | M: r=0.51<br>F: r=0.52 | | | | |
| | | 339 | 155 M; 184 F | 14 y | 13-14 y | 26-30 y | | | M: r=0.52<br>F: r=0.52 | | | | |
| | | 767 | 352 M; 415 F | 8 y | 15-16 y | 20-25 y | | | M: r=0.64<br>F: r=0.53 | | | | |
| | | 568 | 263 M; 305 F | 12 y | 15-16 y | 26-30 y | | | M: r=0.63<br>F: r=0.55 | | | | |
| | | 615 | 299 M; 316 F | 6 y | 17-18 y | 20-25 y | | | M: r=0.72<br>F: r=0.54 | | | | |
| | | 479 | 233 M; 246 F | 11 y | 17-18 y | 26-30 y | | | M: r=0.64<br>F: r=0.48 | | | | |
| Amsterdam Growth & Health Study, the Netherlands | Kemper, 1990[239] | 200 | 93 M; 107 F | 8 y | 13.5±0.6 y | 21.5±0.6 y | No | Pearson's correlation coefficients (no adjustments specified) stratified by sex | M: r=0.70<br>F: r=0.65 | NA | M: r=0.42<br>F: r=0.52 | NA | Examined tracking of TCH/HDL-C ratio (M: r=0.62; F: r=0.68). |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Analyses (adjustments) | Findings | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | | TCH | LDL-C | HDL-C | TG | |
| | | | | | | | | | | | | | determined from non-fasting sample. |
| | Twisk, 1996[240] & 1997[241] | 181 | 83 M; 98 F | 15 y | 13.0±0.8y | 27.1±0.8y | No | Tracking coefficients (TC) estimated using generalized estimating equations & Pearson's correlation coefficients (sex, age, time, change of behaviour – time-dependent | GEE=0.71<br><br>r=0.54 | NA | M: TC=0.51<br>F: TC=0.65<br><br>M: r=0.55<br>F: r=0.62 | NA | Examined tracking of TCH/HDL-C ratio (TC=0.71; r=0.49).<br><br>HDL-C stratified by sex due to significant sex-HDL-C interaction |
| Bogalusa Heart Study, USA | **Webber, 1991[162]** | **1586** | **718 M; 868 F** | **12 y** | **2-14 y** | **14-26 y** | **12 h** | **Spearman correlation coefficients stratified by age, race, sex.** | | | | | Tracking of VLDL-C cholesterol also examined. TCH & LDL-C track better in blacks, HDL-C & triglycerides track better in Whites. |
| | | 669 | 286 M; 383 F | 12 y | 9-14 y | 21-26 y | | | M(White): r=0.45; M(Black): r=0.60; F(White): r=0.42; F(Black): r=0.64 | M(White): r=0.50; M(Black): r=0.69; F(White): r=0.44; F(Black): r=0.61 | M(White): r=0.43; M(Black): r=0.29; F(White): r=0.34; F(Black): r=0.39 | M(White): r=0.42; M(Black): r=0.22; F(White): r=025; F(Black): r=0.11 | |
| | Bao, 1996[231] & Nicklas, 2002[233] | 1169 | Not specified | 15 y | 5-14 y | 20-29 y | 12 h | Spearman correlation coefficients stratified by age, race, sex. Linear regression (age, race, sex, baseline BMI, change in BMI) | r = 0.4-0.6<br><br>$R^2$=0.29 | r = 0.4-0.6<br><br>$R^2$=0.30 | r = 0.2-0.4<br><br>$R^2$=0.27 | r = 0.2-0.4<br><br>$R^2$=0.19 | |
| | Srinivasan, 2006[234] | 1163 | 519 M; 644 F | 27 y | 5-14 y | 32-41 y | 12 h | Linear regression (age, race, sex, | NA | $R^2$=0.23 | NA | NA | Also examined non-HDL-C: $R^2$=0.26 |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Analyses (adjustments) | Findings | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | | TCH | LDL-C | HDL-C | TG | |
| | | | | | | | | baseline BMI, change in BMI | | | | | |
| Cardiovascular Risk in Young Finns Study, Finland | **Porkka, 1991[223]** | **2236** | **1065 M; 1171 F** | **3-6 y** | **3-18 y** | | **12 h** | **Spearman's correlation coefficients stratified by age & sex** | | | | | |
| | | 238 | 104 M; 134 F | 3 y | 18 y | 21 y | | | M: r=0.69<br>F: r=0.51 | M: r=0.69<br>F: r=0.47 | M: r=0.69<br>F: r=0.71 | M: r=0.52<br>F: r=0.57 | Also examined HDL-C /TCH ratio |
| | | 238 | 104 M; 134 F | 6 y | 18 y | 24 y | | | M: r=0.68<br>F: r=0.52 | M: r=0.72<br>F: r=0.49 | M: r=0.58<br>F: r=0.65 | M: r=0.49<br>F: r=0.34 | |
| | | 295 | 238 M; 295 F | 6 y | 15 y | 21 y | | | M: r=0.65<br>F: r=0.57 | M: r=0.69<br>F: r=0.63 | M: r=0.59<br>F: r=0.54 | M: r=0.45<br>F: r=0.31 | |
| | **Porkka, 1994[161]** | **883** | **414 M; 469 F** | **12 y** | **3-18 y** | **15-30 y** | **12 h** | **Spearman correlation coefficients stratified by age & sex** | | | | | Also examined LDL-C/HDL-C ratio |
| | | 156 | 77 M; 79 F | 12y | 9 y | 21 y | | | M: r=0.63<br>F r=0.52 | M: r=0.58<br>F: r=0.53 | M: r=0.56<br>F: r=0.60 | M: r=0.18<br>F: r=0.22 | |
| | | 149 | 64 M; 85 F | 12y | 12 y | 24 y | | | M: r=0.57<br>F r=0.60 | M: r=0.56<br>F: r=0.59 | M: r=0.69<br>F: r=0.52 | M: r=0.45<br>F: r=0.21 | |
| | | 115 | 51 M; 64 F | 12y | 15 y | 27 y | | | M: r=0.40<br>F: r=0.40 | M: r=0.44<br>F: r=0.51 | M: r=0.48<br>F: r=0.57 | M: r=0.53<br>F: r=0.58 | |
| | | 116 | 51 M; 65 F | 12y | 18 y | 30 y | | | M: r=0.73<br>F: r=0.51 | M: r=0.67<br>F: r=0.48 | M: r=0.54<br>F r=0.56 | M: r=0.49<br>F: r=0.37 | |
| Fels Longitudinal Study, USA | Guo, 1993[236] | 96 | Not specified | 12 y | 9-11 y | 19-21 y | Yes | Banded correlation model (none) | r=0.72 | r=0.65 | r=0.46 | NA | |
| Danish Youth & Sport Study, Denmark | Anderson, 1993[237] & 2004[238] | 203 | 88 M; 115 F | 8 y | 15-19 y | 23-27 y | Yes | Pearson's correlation coefficients (none) | M: r=0.39<br>F: r=0.51 | NA | M: r=0.29<br>F: r=0.49 | M: r=0.32<br>F: r=0.33 | Also examined tracking of combined/clustered CHD risk factors |
| Quebec Family Study, Canada | Katzmarzyk, 2001[242] | 147 | 76 M; 71 F | 12 y | 8-18 y | 20-30 y | 12-14 h | Partial correlation coefficients stratified by sex (age at baseline, length of follow-up) | NA | NA | M: r=0.58<br>F: r=0.56 | M: r=0.37<br>F: r=0.20 | Examined tracking of Metabolic syndrome (lipid) components only. |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Analyses (adjustments) | Findings | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | | TCH | LDL-C | HDL-C | TG | |
| Aerobics Centre Longitudinal Study, USA | Eisenmann, 2004[243] | 48 | 36 M; 12 F | 10.9 y | 12-18 y | 26.6±4.9 y | Yes | Partial correlation coefficients (length of follow-up; to account for age & sex; baseline and follow-up variables were regressed onto age & sex and the residuals used for analyses) | r=0.62 | NA | r=0.60 | r=0.54 | Length of follow-up used as covariate since the time between visits varied between participants (range 5-24 y). Also examined tracking of composite risk factor scores. |
| Busselton Study, Australia | **Adams, 2005[244]** | **1764** | **Not specified** | **3-27 y** | **5-18 y** | **19-44 y** | **Not specified** | **Pearson's correlation coefficient (age & survey year)** | | NA | NA | NA | No adjustment for sex, data were combined for track width analysis because variation was not sig different by sex. |
| | | | | 15-20 y | 5-9 y | 20-36 y | | | r=0.45 | | | | |
| | | | | >20 y | | | | | r=0.44 | | | | |
| | | | | 10-15 y | 10-14 y | 20-41 y | | | r=0.35 | | | | |
| | | | | 15-20 y | | | | | r=0.44 | | | | |
| | | | | >20 y | | | | | r=0.42 | | | | |
| | | | | 3 y | 15-18 y | 20-45 y | | | r=0.49 | | | | |
| | | | | 5-10 y | | | | | r=0.39 | | | | |
| | | | | 10-15 y | | | | | r=0.43 | | | | |
| | | | | 15-20 y | | | | | r=0.48 | | | | |
| | | | | >20 y | | | | | r=0.40 | | | | |

TCH = total cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very-low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; M = males; F = female; NA = not available; CHD = coronary heart disease; MetS = metabolic syndrome; BMI = body mass index; GEE = generalized estimating equations.

# APPENDIX 8

## Overview of studies of blood lipid and lipoprotein tracking from childhood to adulthood: stability data

**Table**. Overview of studies that have examined tracking of lipid and lipoprotein levels by risk-group from childhood and adolescence to adulthood, sorted by date of first publication and study

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Risk group | | Proportion in same risk group at follow-up | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | Baseline | Follow-up | | |
| Beaver County Lipid Study, USA | Orchard, 1983[235] | 561 | 264 M; 297 F | 9 y | 11-14 y | 20-24 y | 10 h | TCH ≥80th percentile | TCH ≥80th percentile | M=50.0%; F=46.8% | |
| | Stuhldreher, 1991[224] | 295 | 144 M; 155 F | 16 y | 11-14 y | 27-30 y | 10 h | TCH ≥80th percentile | TCH ≥80th percentile | M=37.9%; F=42.4% | |
| Muscatine Study, USA | **Lauer, 1988[160]/1989[232]** | **2446** | **1167 M; 1279 F** | **6-15 y** | **8-18 y** | **20-30 y** | **12 h** | TCH≥90th percentile | TCH≥90th percentile | 24-32% | TCH only. Single measurement at baseline considered for risk classification |
| | | | | | | | | TCH≥90th percentile | TCH≥75th percentile | 50-87% | |
| | | | | | | | | TCH<90th percentile | TCH<75th percentile | 78-84% | |
| | Lauer, 1990[245] | 2367 | 1133 M; 1234 F | 6-15 y | **8-18 y** | **20-30 y** | 12 h | TCH≥75th percentile | TCH≥6.20 mmol/L | 17% | Baseline risk group defined from two consecutive TCH levels ≥75th percentile |
| | | | | | | | | TCH≥90th percentile | TCH≥6.20 mmol/L | 34% | Follow-up risk groups defined using original NCEP adult guidelines.[338] (equivalent to current) |
| | | | | | | 20-25 | | TCH≥75th percentile | TCH≥5.17 mmol/L | M=44.6% F=57.4% | |
| | | | | | | | | TCH≥90th percentile | TCH≥5.17 mmol/L | M=75.0% F=75.0% | |
| | | | | | | 26-30 | | TCH≥75th percentile | TCH≥5.17 mmol/L | M=68.0% F=48.5% | |
| | | | | | | | | TCH≥90th percentile | TCH≥5.17 mmol/L | M=77.8% F=55.6% | |
| Amsterdam Growth & Health Study, the Netherlands | Kemper, 1990[239] | 200 | 93 M; 107 F | 8 y | 13.5±0.6 y | 21.5±0.6 y | No | TCH ≥75th percentile | TCH ≥75th percentile | M=61% F=42% | Blood lipids determined from non-fasting sample. |
| | | | | | | | | HDL-C <25th percentile | HDL-C <25th percentile | M=53% F=48% | Examined TCH/HDL-C ratio (M=54%; F=63%). |
| | Twisk, 1997[241] | 181 | 83 M; 98 F | 15 y | 13.0±0.8y | 27.1±0.8y | No | | | | Not available – predictive values not calculated as all available longitudinal data (multiple follow- |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Risk group | | Proportion in same risk group at follow-up | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | Baseline | Follow-up | | |
| | | | | | | | | | | | ups) used in analyses. |
| Bogalusa Heart Study, USA | **Webber, 1991[162]** | **1586** | **718 M; 868 F** | **12 y** | **2-14 y** | **14-26 y** | **12 h** | | | | |
| | | 669 | 286 M; 383 F | | 9-14 y | 21-26 y | | TCH≥75th percentile | TCH≥75th percentile | M(white)=47%; M(black)=68%; F(white)=40%; F(black)=59% | Tracking of VLDL-C also examined: M(white)=50%; M(black)=36%; F(white=45%; F(black)=30% |
| | | | | | | | | LDL-C ≥75th percentile | LDL-C ≥75th percentile | M(white)=53%; M(black)=69%; F(white)=52%; F(black)=55% | |
| | | | | | | | | HDL-C <25th percentile | HDL-C <25th percentile | M(white)=42%; M(black)=53%; F(white)=46%; F(black)=38% | |
| | | | | | | | | TG≥75th percentile | TG≥75th percentile | M(white)=57%; M(black)=33%; F(white)=38%; F(black)=24% | |
| | Bao, 1996[231] & Nicklas, 2002[233] | 1169 | Not specified | 15 y | 5-14 y | 20-29 y | 12 h | TCH, LDL-C, HDL-C, TG ≥80th percentile | TCH, LDL-C, HDL-C, TG ≥80th percentile | TCH & LDL-C >40%; HDL-C & TG >30% | Used pediatric[196] & adult[412] NCEP LDL-C cut-points to define risk groups. Examined effect of repeated measures on misclassification. |
| | | | | | | | | LDL-C ≥3.36 mmol/L (≥130 mg/dL) | LDL-C ≥4.14 mmol/L (≥160 mg/dL | 28% | |
| | | | | | | | | LDL-C ≥90th percentile | LDL-C ≥4.14 mmol/L (≥160 mg/dL) | single LDL-C measure in childhood=26.9%; two LDL-C measures in childhood=52.3% | |
| | Srinivasan, 2006[234] | 1163 | 519 M; 644 F | 27 y | 5-14 y | 32-41 y | 12 h | LDL-C ≥80th percentile | LDL-C ≥80th percentile | ~38% | Also examined non-HDL-C = 38.5% |
| | | | | | | | | LDL-C ≥3.36 mmol/L (≥130 mg/dL) | LDL-C ≥4.14 mmol/L (≥160 mg/dL) | 41.8% | |
| Cardiovascular Risk in Young Finns Study, Finland | **Porkka, 1991[223]** | **2236** | **1065 M; 1171 F** | **3-6 y** | **3-18 y** | | **12 h** | TCH ≥80th percentile | TCH ≥80th percentile | 3-year, M=65.6%; F=55.3% 6-year, M=55.5%; F=50.4% | Also examined HDL-C/TCH ratio (3-year, M=66.0%; F=61.9% 6-year, M=54.0%; |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Risk group | | Proportion in same risk group at follow-up | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | Baseline | Follow-up | | |
| | | | | | | | | | | | F=56.8%) |
| | | | | | | | | LDL-C ≥80th percentile | LDL-C ≥80th percentile | 3-year, M=59.8%; F=56.3%<br>6-year, M=58.9%; F=54.5% | |
| | | | | | | | | HDL-C <20th percentile | HDL-C <20th percentile | 3-year, M=60.6%; F=53.5%<br>6-year, M=54.1%; F=49.0% | |
| | | | | | | | | TG ≥80th percentile | TG ≥80th percentile | 3-year, M=42.4%; F=41.8%<br>6-year, M=39.4%; F=34.2% | |
| | **Porkka, 1994[161]** | **883** | **414 M; 469 F** | **12 y** | **3-18 y** | **15-30 y** | **12 h** | TCH, LDL-C, HDL-C, TG ≥80th percentile | TCH, LDL-C, HDL-C, TG ≥80th percentile | TCH, LDL-C, & HDL-C ~50%; TG ~30% | Examined effects of repeated measurements & regression-toward-the-mean. Repeat measurements increased the amount of adult lipid variability explained by up to 50% in TCH, LDL-C & HDL-C models. |
| Fels Longitudinal Study, USA | Guo, 1993[236] | 96 | Not specified | 12 y | 9-11 y | 19-21 y | Yes | TCH ≥5.17 mmol/L (≥130 mg/dL) | TCH ≥5.17 mmol/L (≥160 mg/dL) | M=59%<br>F=59% | Cut-points used at baseline are to NCEP high-risk;[196] cut-points used at follow-up are equivalent to borderline-risk (TCH & LDL-C), and high-risk (HDL-C) according to NCEP[304] |
| | | | | | | | | LDL-C ≥3.36 mmol/L (≥130 mg/dL) | LDL-C ≥3.36 mmol/L (≥160 mg/dL) | M=46%<br>F=46% | |
| | | | | | | | | HDL-C <0.91 mmol/L (<35 mg/dL) | HDL-C <1.03 mmol/L (<40 mg/dL) | M=30%<br>F=25% | |
| Danish Youth & Sport Study, Denmark | Anderson, 1993[237] | 203 | 88 M; 115 F | 8 y | 15-19 y | 23-27 y | Yes | TCH ≥80th percentile | TCH ≥80th percentile | M=26%<br>F=62% | Also examined TCH/HDL-C ratio: M=47%; F=58% |
| | | | | | | | | 1/HDL-C ≥80th percentile | 1/HDL-C ≥80th percentile | M=47%<br>F=58% | |
| | | | | | | | | TG ≥80th percentile | TG ≥80th percentile | M=50%<br>F=29% | |
| Busselton Study, | **Adams, 2005[244]** | **1764** | **Not** | **3-27 y** | **5-18 y** | **19-44 y** | **Not** | TCH ≥75th | TCH ≥75th | 40-60% | 15-18 y at first measurement |

| Study | Publication | N | Population | Duration of follow-up | Age | | Fasting status | Risk group | | Proportion in same risk group at follow-up | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Baseline | Follow-up | | Baseline | Follow-up | | |
| Australia | | | **specified** | | | | **specified** | percentile | percentile | | maintained rank in extreme quarter more consistently than those <15 y |

TCH = total cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very-low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; M = males; F = female; NA = not available; NCEP = National Cholesterol Education Program

## APPENDIX 9

**Area under ROC curve point estimates and 95% confidence intervals for prediction of abnormal lipid and lipoprotein levels in adults using NCEP and NHANES adolescent lipoprotein classifications for Young Finns with repeat lipid and lipoprotein measurements**

**Table.** Area under ROC curve point estimates and 95% confidence intervals for prediction of abnormal lipid and lipoprotein levels in adults using NCEP and NHANES adolescent lipoprotein classifications for Young Finns with repeat lipid and lipoprotein measurements

| | | NCEP | | NHANES | |
|---|---|---|---|---|---|
| Lipoprotein | n | AUC (95% CI) | P value* | AUC (95% CI) | P value* |
| Total cholesterol | | | | | |
| Single measurement | 682 | 0.71 (0.67-0.75) | | 0.71 (0.66-0.75) | |
| Repeat measurements† | 682 | 0.76 (0.72-0.80) | <0.01 | 0.75 (0.70-0.79) | <0.01 |
| LDL cholesterol | | | | | |
| Single measurement | 667 | 0.74 (0.70-0.78) | | 0.74 (0.70-0.78) | |
| Repeat measurements† | 667 | 0.77 (0.72-0.81) | 0.03 | 0.76 (0.71-0.80 | 0.13 |
| HDL cholesterol | | | | | |
| Single measurement | 679 | 0.70 (0.67-0.74) | | 0.74 (0.70-0.78) | |
| Repeat measurements† | 679 | 0.77 (0.74-0.81) | <0.01 | 0.80 (0.76-0.84) | <0.01 |
| Triglycerides | | | | | |
| Single measurement | 682 | 0.59 (0.54-0.65) | | 0.54 (0.51-0.58) | |
| Repeat measurements† | 682 | 0.72 (0.66-0.78) | <0.01 | 0.61 (0.56-0.67) | <0.01 |

* Test for difference between AUCs of single and repeat measurements, †12 & 15 year old Finns at baseline (1980) who had repeat lipoprotein measurements as adolescents in 1983.

Abbreviations: AUC, area under the receiver-operating characteristic curve; CI, confidence interval

# APPENDIX 10

## Summary of studies showing the association between carotid IMT and clinical cardiovascular end points

**Table.** Carotid IMT and clinical cardiovascular disease end points: Overview of population-based cohort studies, sorted by date of first publication and study

| | | | | | | | IMT Definition | | Analyses | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study | Publication | Design | Population | End Point/s | Sample size at risk/Events | Follow-up | Segments | Measurements | IMT Modelled As | Adjusted for | Findings |
| KIHD (Kuopio Ischemic Heart Disease Risk Factor Study), Eastern Finland | Salonen, 1993[413] | Longitudinal | Men 42 to 60 y | Definite or possible MI | 1257/36 | 1 mo-3 y | Left & right CCA | Mean, far wall | Continuous; fixed cut-point (IMT >1.0 mm); Cox proportional hazards | Unadjusted (effect size for adjusted model not published) | Risk of MI increased by 11% (6%-16%) for each 0.1 mm of IMT. Those beyond cut-point: HR=2.1 (0.8-5.2) for MI |
| ARIC (Atherosclerosis Risk in Communities Study), USA | Chambless, 1997[414] | Longitudinal | 45-64 y free of clinical CHD event | MI | 10,841/290 or 13,204/626* | Median: 5.2 y or 10.6 y* | Left & right CCA, BIF, ICA, combined | Mean, far wall | Continuous, thirds, 95th percentile, fixed cut-points (IMT >1.0 mm); Cox proportional hazards | Age, centre, race, LDL & HDL cholesterol, BMI, sports activity, cigarette-years, hypertension, diabetes, ethanol, fibrinogen, stratified by sex | For each SD (0.19 mm) increment of mean combined IMT: Male, HR=1.2 (1.0-1.3) Female, HR=1.4 (1.2-1.6). Upper vs. lower third: Male, HR=2.0 (1.3-3.1), Female, HR=3.8 (1.7-8.4) |
| | Chambless, 2000[415] | Longitudinal | 45-64 y without previous stroke | Stroke | 14,214/199 or 14,165/371* | Median: 7.2 y or 10.7 y* | Left & right CCA, BIF, ICA, combined | Mean, far wall | Continuous, thirds, 95th percentile, fixed cut-points; Cox proportional hazards | Age, centre, race, LDL & HDL cholesterol, BMI, waist:hip ratio, sports activity, current smoking, former smoking, hypertension, diabetes, left ventricular hypertrophy, fibrinogen, white blood cell count, stratified by sex | For each SD (0.18 mm) increment in mean combined IMT: Male, HR=1.2 (1.1-1.4) Female, HR=1.4 (1.2-1.6). Upper vs. lower third: Male, HR=2.2 (1.3-4.0), Female, HR=2.3 (1.1-4.9) |
| Rotterdam Study, the Netherlands | Bots, 1997[416] | Nested case-control | ≥ 55 y | MI, Stroke | Cases: 98 (MI), 95 (stroke); Controls: 1373 | Mean: 2.7 y | Left & right CCA | Mean, near + far wall | Continuous, fifths; logistic | Age, sex, BMI, smoking, systolic blood pressure, | For each SD (0.16 mm) increment of |

| Study | Publication | Design | Population | End Point/s | Sample size at risk/Events | Follow-up | IMT Definition | | Analyses | | Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Segments | Measurements | IMT Modelled As | Adjusted for | |
| | | | | | | | | | regression | hypertension, total & HDL cholesterol, diabetes, inclusion/exclusion previous MI & stroke | mean CCA IMT: MI, OR=1.4 (1.2-1.8); stroke, OR=1.5 (1.2-1.8); Upper quintile vs. reference category: MI, OR=1.4 (0.7-3.1); stroke, OR=2.8 (1.2-6.6) for MI |
| | Iglesias del Sol, 2002[417] | Case-cohort | ≥ 55 y | MI | Cases: 194; Controls: 2073 | Mean: 4.6 y | Left & right CCA, BIF, ICA, combined | mean max, near + far wall | Continuous, quarters; Cox proportional hazards | Age, sex, BMI, smoking, systolic & diastolic blood pressure, total & HDL cholesterol, diabetes, inclusion/exclusion previous MI & stroke | For each SD (NA) increment in combined IMT: MI, RR=1.4 (1.2-1.6); Upper vs. lower quarter: MI, RR=4.8 (2.5-9.4) |
| | Hollander, 2003[418] | Longitudinal | ≥ 55 y | Stroke | 5479/378 | Mean: 6.1 y | Left & right CCA | Mean, near + far wall | Continuous, thirds; Cox proportional hazards | Age, sex, smoking, systolic & diastolic blood pressure, total & HDL cholesterol, diabetes, history of CVD | For each SD increment (NA) in IMT: stroke, RR=1.2 (1.0-1.4); Upper vs. lower third: stroke, RR=2.3 (1.3-3.9) |
| CHS (Cardiovascular Health Study), USA | O'leary, 1999[419] | Longitudinal | ≥ 65 y without CVD | MI, stroke | 4476/267 (MI), 284 (stroke) | Median: 6.2 y | Left & right CCA, ICA, combined | Mean max, near + far wall | Continuous, fifths; Cox proportional hazards | Age, sex, systolic & diastolic blood pressure, atrial fibrillation, pack-years of smoking, diabetes | For each SD (NA) increment in combined IMT: RR=1.4 (1.3-1.5); MI, RR=1.4 (1.3-1.5); stroke, RR=1.3 (1.2-1.5); Upper vs. lower fifth: combined end-point, RR=3.2 (2.2-4.5); MI, RR=3.6 (2.1- |

| Study | Publication | Design | Population | End Point/s | Sample size at risk/Events | Follow-up | IMT Definition | | Analyses | | Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Segments | Measurements | IMT Modelled As | Adjusted for | |
| | | | | | | | | | | | 6.1); stroke, RR=2.6 (1.6-4.0) |
| | Cao, 2007[420] | Longitudinal | ≥ 65 y without CVD | MI, stroke, CVD death, combined, All-cause mortality | 5020/593 (MI), 613 (stroke), 696 (CVD death), 1844 (all-cause death) | Median: 11.0 y | Left & right CCA, ICA, combined | Mean max, near + far wall | Thirds; Cox proportional hazards | Age, sex, race, systolic & diastolic blood pressure, BMI, smoking, amount smoked, HDL & LDL cholesterol, diabetes, CRP | Upper vs. lower third, MI: HR=1.8 (1.4-2.4), stroke: HR=1.8 (1.4-2.3), CVD death, HR=2.2 (1.7-2.8), composite CVD: HR=1.8 (1.5-2.2), all-cause mortality: HR=1.5 (1.3-1.8) |
| No acronym, Japan | Kitamura, 2004[421] | Longitudinal | Men 60-74 y without previous stroke or CHD | Stroke | 1289/34 | Mean: 4.5 y | Left & right CCA, ICA (incl. BIF), combined | Max, near + far wall | Quarters; Cox proportional hazards | Age, systolic blood pressure, BMI, antihypertensive medication, ST-T abnormalities, community | Upper vs. lower quarter, stroke: RR=4.8 (1.9-12.0) |
| MDCS (Malmö Diet and Cancer Study), Sweden | Rosvall, 2005 (a)[422] | Longitudinal | 46-68 y without previous stroke or cardiovascular disease | MI or cardiac death | 5163/113 | Median: 7 y | Right CCA | Mean, far wall | Continuous, thirds, fixed cut-points (several); Cox proportional hazards | Age, sex, low physical activity, smoking habits, hypertension, diabetes, LDL & HDL cholesterol, waist circumference | For each SD (0.15 mm) increment in CCA IMT: MI/cardiac death, HR=1.2 (1.1-1.4); Upper vs. lower third: RR=1.5 (0.8-2.6) |
| | Rosvall, 2005 (b)[423] | Longitudinal | 46-68 y without previous stroke or cardiovascular disease | Stroke | 5163/86 | Median: 7 y | Right CCA | Mean, far wall | Continuous, thirds, fixed cut-points; Cox proportional hazards | Age, sex, low physical activity, smoking habits, systolic blood pressure, hypertension, diabetes, LDL & HDL cholesterol, triglycerides, waist circumference | For each SD (0.15 mm) increment in CCA IMT: stroke, HR=1.2 (1.0-1.4); Upper vs. lower third: RR=2.5 (1.2-5.4) |
| LILAC (Longitudinal Investigation for the Longevity | Murakami, 2005[424] | Longitudinal | > 75 y | All-cause and vascular mortality | 298/30 (death), 9 (cardiovascular death) | Mean: 3.2 y | Left & right CCA, BIF, ICA | Mean, near + far wall† | Continuous; Cox proportional hazards | Age, Mini-Mental State Examination Score | For each 0.1 mm increment in left CCA IMT, all-cause mortality, |

| Study | Publication | Design | Population | End Point/s | Sample size at risk/Events | Follow-up | IMT Definition | | Analyses | | Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Segments | Measurements | IMT Modelled As | Adjusted for | |
| and Aging in Hokkaido County), Japan | | | | | | | | | | | RR=1.2 (1.0-1.4); cardiovascular mortality, RR=1.3 (1.0-1.8). For each 0.1 mm increment in right CCA IMT, all-cause mortality, RR=1.5 (1.1-2.0); cardiovascular mortality, RR=1.4 (1.0-2.0). |
| CAPS (Carotid Atherosclerosis Progression Study), Germany | Lorenz, 2006[425] | Longitudinal | 19-90 y | MI, stroke | 5052/228 (myocardial event), 107 (stroke or TIA), 50 (death) | Mean: 4.2 y | Left & right CCA | Mean, far wall | Continuous, quarters, Cox proportional hazards | Age, sex, BMI, systolic & diastolic blood pressure, antihypertensive medication, LDL cholesterol, lipid-lowering medication, nicotine consumption, diabetes | For each SD (0.16 mm) increment in CCA IMT: combined end-point, HR=1.2 (1.1-1.3); MI, RR=1.2 (1.1-1.3); stroke, RR=1.1 (1.0-1.3); Upper vs. lower quarter: combined end-point, RR=1.9 (1.1-3.2); MI, RR=1.8 (1.0-3.5); stroke, RR=1.8 (0.6-5.2) |
| The Edinburgh Artery Study, UK | Price, 2007[426] | Longitudinal | 60-79 y free of MI or stroke | MI, stroke | 1007/78 (MI), 65 (stroke) | 12 y (mean/median not specified) | Left & right CCA | Max, far wall | fixed cut-point (IMT ≥0.9mm), logistic regression | Age, sex, diabetes, smoking, systolic blood pressure, total/HDL cholesterol | Above vs. below cut-point, OR (MI/stroke) = 1.6 (1.1-2.4) |
| The Tromsø Study, Norway | Johnsen, 2007[427] | Longitudinal | 25-84 y with no previous MI | MI | 6226/295 | Median: 5.8 y | Right CCA, combined CCA, | Mean IMT, near + far wall | Quarters, Cox proportional hazards | Total & HDL cholesterol, smoking, systolic blood pressure, white blood | Upper vs. lower quarter, Male: RR=1.7 (1.0-3.1), |

| Study | Publication | Design | Population | End Point/s | Sample size at risk/Events | Follow-up | IMT Definition | | Analyses | | Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Segments | Measurements | IMT Modelled As | Adjusted for | |
| | | | | | | | BIF, ICA | | | cell count, monocyte count, fibrinogen, lipid-lowering & antihypertensive medication | Female: RR=2.9 (1.1-7.7) |

*Additional data incorporated in the Lorenz et al. systematic review and meta-analysis.

†IMT definition not clearly described.

MI = myocardial infarction; CVD = cardiovascular disease; CHD = coronary heart disease; CCA = common carotid artery; BIF = carotid bifurcation; ICA = internal carotid artery; HR = hazards ratio; RR = relative risk; OR = odds ratio; SD = standard deviation. Studies included in the Lorenz et al.[375] systematic review and meta-analysis are highlighted in blue.

## Appendix 11

**Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) data of NCEP and NHANES classifications for adolescent borderline risk and high risk lipoprotein variable cut-points to predict high IMT in adulthood by cohort**

**Table**. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) data of NCEP and NHANES classifications for adolescent borderline risk and high risk lipoprotein variable cut-points to predict dyslipidemia in adulthood

| | | | Dyslipidemia status in adolescence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Borderline risk | | | | High risk | | | | |
| | | | Sensitivity | Specificity | PPV | NPV | Sensitivity | Specificity | PPV | NPV | AUC |
| LDL cholesterol (Normal weight) | Young Finns | NCEP | 74.8 | 32.1 | 11.9 | 91.2 | 45.4 | 60.0 | 12.3 | 89.9 | 0.54 |
| | | NHANES | 59.7 | 46.1 | 12.0 | 90.3 | 27.7 | 75.0 | 12.0 | 89.4 | 0.54 |
| | Bogalusa | NCEP | 9.1 | 85.1 | 3.8 | 93.5 | 0.0 | 94.1 | 0.0 | 93.5 | 0.47 |
| | | NHANES | 0.0 | 89.9 | 0.0 | 93.2 | 0.0 | 96.4 | 0.0 | 93.6 | 0.50 |
| | CDAH | NCEP | 46.9 | 64.7 | 16.1 | 89.4 | 28.1 | 87.3 | 24.3 | 89.4 | 0.58 |
| | | NHANES | 34.4 | 77.4 | 18.0 | 89.1 | 15.6 | 94.1 | 27.8 | 88.5 | 0.56 |
| | Pooled | NCEP | 64.8 | 44.0 | 12.2 | 91.3 | 38.9 | 68.7 | 12.9 | 90.4 | 0.55 |
| | | NHANES | 50.6 | 56.6 | 12.2 | 90.6 | 23.5 | 80.7 | 12.7 | 89.8 | 0.55 |
| LDL cholesterol (Overweight/obese) | Young Finns | NCEP | 88.2 | 30.4 | 23.8 | 91.3 | 70.6 | 53.6 | 27.3 | 88.1 | 0.63 |
| | | NHANES | 76.5 | 40.6 | 24.1 | 87.5 | 47.1 | 76.8 | 66.7 | 85.5 | 0.65 |
| | Bogalusa | NCEP | 41.2 | 66.7 | 28.0 | 78.3 | 35.3 | 83.3 | 40.0 | 80.4 | 0.56 |
| | | NHANES | 41.2 | 72.2 | 31.8 | 79.6 | 23.5 | 90.7 | 44.4 | 79.0 | 0.61 |
| | CDAH | NCEP | 50.0 | 75.9 | 22.2 | 91.7 | 25.0 | 86.2 | 20.0 | 89.3 | 0.63 |
| | | NHANES | 25.0 | 79.3 | 14.3 | 88.5 | 25.0 | 96.6 | 50.0 | 90.3 | 0.60 |
| | Pooled | NCEP | 63.2 | 52.0 | 24.7 | 84.9 | 50.0 | 70.4 | 29.7 | 84.9 | 0.60 |
| | | NHANES | 55.3 | 59.2 | 25.3 | 84.1 | 34.2 | 85.5 | 37.1 | 83.4 | 0.63 |

| | | | Dyslipidemia status in adolescence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Borderline risk | | | | High risk | | | | |
| | | | Sensitivity | Specificity | PPV | NPV | Sensitivity | Specificity | PPV | NPV | AUC |
| HDL cholesterol (Normal weight) | Young Finns | NCEP | 51.3 | 47.1 | 10.7 | 88.7 | 0.8 | 99.5 | 16.7 | 89.1 | 0.49 |
| | | NHANES | 47.9 | 49.9 | 10.5 | 88.6 | 2.5 | 97.3 | 10.3 | 89.0 | 0.49 |
| | Bogalusa | NCEP | 63.6 | 38.5 | 6.3 | 94.2 | 9.1 | 92.9 | 7.7 | 94.0 | 0.52 |
| | | NHANES | 63.6 | 46.8 | 33.3 | 92.9 | 9.1 | 87.0 | 33.3 | 79.3 | 0.53 |
| | CDAH | NCEP | 59.4 | 33.9 | 11.5 | 85.2 | 9.4 | 100.0 | 100.0 | 88.4 | 0.50 |
| | | NHANES | 65.6 | 34.4 | 12.7 | 87.4 | 12.5 | 93.7 | 22.2 | 88.1 | 0.52 |
| | Pooled | NCEP | 53.7 | 43.9 | 10.3 | 88.8 | 3.1 | 98.8 | 22.7 | 89.5 | 0.49 |
| | | NHANES | 52.5 | 47.0 | 10.6 | 89.2 | 4.9 | 95.4 | 11.4 | 89.4 | 0.49 |
| HDL cholesterol (Overweight/obese) | Young Finns | NCEP | 94.1 | 26.1 | 23.9 | 94.7 | 5.9 | 94.2 | 20.0 | 80.2 | 0.60 |
| | | NHANES | 100.0 | 26.1 | 25.0 | 100.0 | 5.9 | 88.4 | 11.1 | 79.2 | 0.59 |
| | Bogalusa | NCEP | 88.2 | 46.4 | 33.3 | 92.9 | 29.4 | 82.1 | 33.3 | 79.3 | 0.67 |
| | | NHANES | 76.5 | 44.6 | 29.5 | 86.2 | 41.2 | 76.8 | 35.0 | 81.1 | 0.63 |
| | CDAH | NCEP | 100.0 | 10.3 | 13.3 | 100.0 | 0.0 | 96.6 | 0.0 | 87.5 | 0.53 |
| | | NHANES | 100.0 | 13.8 | 13.8 | 100.0 | 50.0 | 86.2 | 33.3 | 92.6 | 0.72 |
| | Pooled | NCEP | 92.1 | 30.5 | 24.6 | 94.0 | 15.8 | 90.3 | 28.6 | 81.3 | 0.62 |
| | | NHANES | 89.5 | 30.5 | 24.1 | 92.2 | 26.3 | 83.8 | 28.6 | 82.2 | 0.62 |

Sensitivity = true positives/(true positives + false negatives) X 100. Specificity = true negatives/(true negatives + false positives) X 100. PPV = true positives/(true positives + false positives) X 100. NPV = true negatives/(true negatives + false negatives) X 100

## ORIGINAL COMMUNICATIONS